Maastricht University

A high tumour-stroma ratio (TSR) in colon tumours and its metastatic lymph nodes predicts poor cancer-free survival and chemo resistance

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

Download date: 04 Dec. 2022

RESEARCH ARTICLE

A high tumour-stroma ratio (TSR) in colon tumours and its metastatic lymph nodes predicts poor cancer-free survival and chemo resistance

M. T. A. Strous[1,2] · T. K. E. Faes[3] · A. L. H. M. Gubbels[3] · R. L. A. van der Linden[4] · W. E. Mesker[5] · K. Bosscha[4] · C. M. Bronkhorst[6] · M. L. G. Janssen-Heijnen[2,7] · F. J. Vogelaar[1] · A. P. de Bruïne[3]

Abstract

Purpose Despite known high-risk features, accurate identification of patients at high risk of cancer recurrence in colon cancer remains a challenge. As tumour stroma plays an important role in tumour invasion and metastasis, the easy, low-cost and highly reproducible tumour-stroma ratio (TSR) could be a valuable prognostic marker, which is also believed to predict chemo resistance.

Methods Two independent series of patients with colon cancer were selected. TSR was estimated by microscopic analysis of 4 μm haematoxylin and eosin (H&E) stained tissue sections of the primary tumour and the corresponding metastatic lymph nodes. Patients were categorized as TSR-low (≤ 50%) or TSR-high (> 50%). Differences in overall survival and cancer-free survival were analysed by Kaplan–Meier curves and cox-regression analyses. Analyses were conducted for TNM-stage I–II, TNM-stage III and patients with an indication for chemotherapy separately.

Results We found that high TSR was associated with poor cancer-free survival in TNM-stage I–II colon cancer in two independent series, independent of other known high-risk features. This association was also found in TNM-stage III tumours, with an additional prognostic value of TSR in lymph node metastasis to TSR in the primary tumour alone. In addition, high TSR was found to predict chemo resistance in patients receiving adjuvant chemotherapy after surgical resection of a TNM-stage II–III colon tumour.

Conclusion In colon cancer, the TSR of both primary tumour and lymph node metastasis adds significant prognostic value to current pathologic and clinical features used for the identification of patients at high risk of cancer recurrence, and also predicts chemo resistance.

Keywords Colon cancer · Tumour stroma ratio · TSR · Survival · Chemo resistance

Introduction

Chances of survival of colorectal cancer differ widely among patients. In current clinical practice, the indication for post-operative systemic treatment is still under debate and is mainly based on pathological staging of the tumour classified by the tumour-node-metastasis (TNM) classification of the American Joint Committee on Cancer (AJCC) [1, 2]. In this, nodal involvement is the most important factor in the identification of patients at high risk of cancer recurrence.

✉ M. T. A. Strous
  maudstrous@viecuri.nl

1 Department of Surgery, VieCuri Medical Centre, Tegelseweg 210, 5912 BL Venlo, The Netherlands

2 Department of Epidemiology, GROW School for Oncology and Developmental Biology, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, The Netherlands

3 Department of Pathology, VieCuri Medical Centre, Venlo, The Netherlands

4 Department of Surgery, Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands

5 Department of Pathology, Leiden University Medical Centre, Leiden, The Netherlands

6 Department of Pathology, Jeroen Bosch Hospital, 's-Hertogenbosch, The Netherlands

7 Department of Epidemiology, VieCuri Medical Center, Venlo, The Netherlands

For these patients, adjuvant chemotherapy would be indicated. However, lymph node involvement alone proves to be inadequate to assess the metastatic potential of a tumour. In 15–20% of all patients with a TNM stage I-II colon tumour, a tumour assumed to be of low metastatic potential, cancer recurs within 5 years after treatment [3, 4]. The American Society of Clinical Oncology (ASCO) attempted to identify high-risk patients within this node negative group with high-risk features, e.g. T4 stage, bowel perforation or clinical bowel obstruction, inadequate lymph node sampling, poorly differentiated histology or lymph-angio invasion [5]. However, these features have been shown insufficient for accurate identification of patients with an indication for adjuvant chemotherapy [6]. To be able to identify patients at risk of cancer recurrence more accurately, and prevent under- and overtreatment, additional prognostic markers are needed.

Recent findings suggest that the tumour microenvironment plays an important role in tumour invasion and metastasis [7, 8]. This tumour microenvironment, referred to as tumour-stroma, consists mainly of myofibroblasts, an activated form of fibroblasts [2]. Activation of fibroblasts is mediated by growth factors as TGF-β and platelet-derived growth factor (PDGF), cytokines, and metabolites released by cancer cells [9–11]. Myofibroblasts regulate a number of tumour-promoting functions. By activating the Wnt pathway they stimulate the preservation of cancer stem cells at the invasive front of the tumour and promote epithelial-to-mesenchymal (EMT) transition which is suggested to cause epithelial tumour cells to change to a mesenchymal cell phenotype and thereby form more myofibroblasts [2, 7, 12]. Studies have shown that myofibroblasts promote tumour invasion by secreting soluble factors as Hepatocyte Growth Factor (HGF) and Secreted Protein Acidic and Rich in Cysteine (SPARC), and by remodelling the extracellular matrix, metalloproteinases and their inhibitors, produced by both cancer and stromal cells [7, 13]. Myofibroblasts, or cancer-associated fibroblasts, are known to express the cell surface protease Fibroblast Associated Protein (FAP), especially at the invasive part of the tumour. This protein contributes to the invasive behaviour of cancer cells by suppressing the anti-tumour immune response [14, 15]. It is also involved in remodelling of the extracellular matrix, which facilitates tumour migration [16]. The tumour-stroma itself impacts the aggressive behaviour of cancer cells through autocrine- and paracrine signalling, and mechanical pressure. The stroma forms a physical barrier around the tumour that increases the interstitial pressure and hypoxia in the tumour. Cancer cells respond to hypoxic conditions through the up-regulation of hypoxia-inducible factor 1α, a master transcription factor that activates a whole range of genes involved in angiogenesis, migration, metabolism, tumour invasion and metastasis [17]. It has been suggested that this growth stimulating micro-environment not only promotes tumour invasion and metastasis, but also chemotherapy resistance of the tumour [18].

Multiple tumour microenvironment derived parameters have been suggested in literature, but few have been implemented in clinical practice. To make a parameter implementable in the pathologist's routine diagnostics, its assessment should be simple, reliable and cost-effective. Tumour-stroma ratio (TSR), as described by Mesker et al., seems to comply with these requirements [19]. With this method, the tumour microenvironment is assessed by standard microscopical analysis on conventional haematoxylin and eosin (H&E)-stained sections of the most invasive part of the tumour. A high stromal content, as estimated using this method, has been associated with poorer survival in a number of solid cancers, including colorectal cancer [19–21]. Adding to this, tumour-stroma scoring in metastatic lymph nodes has been suggested to predict survival even more accurately [22, 23].

With this study we aim to assess the prognostic value of tumour-stroma in colon tumours and their lymph node metastases on oncological outcome, while using the scoring technique described in detail by van Pelt et al., and estimate its reproducibility [24]. In addition, we aimed to determine whether high tumour-stroma content in colon tumours is associated with chemo resistance in patients receiving adjuvant chemotherapy.

Methods

Patients and data

The original series included all consecutive patients diagnosed with a TNM-stage I–III primary colon carcinoma, and treated by complete oncological resection between January 2010 up to and including December 2016 at VieCuri Medical Centre. The following patients were excluded: patients with carcinoma in situ, patients with metastatic disease at time of surgery or metastasis within 3 months after surgery as this was considered present at time of surgery, patients with a neuroendocrine tumour because of different tumour characteristics and prognosis, all rectal tumours because of differences in tumour biology and treatment, and patients of whom tumour tissue was missing or insufficient for reassessment. To confirm the findings found in this original series, we reproduced this study in an independent study cohort. An existing cohort of all consecutive patients diagnosed with a TNM-stage I–II primary colon carcinoma, and treated by complete oncological resection from November 2002 up to and including December 2012 at Jeroen Bosch Hospital was used as validation series. Exclusion criteria covenant those of the original series. A flow chart of the study is presented in Fig. 1.
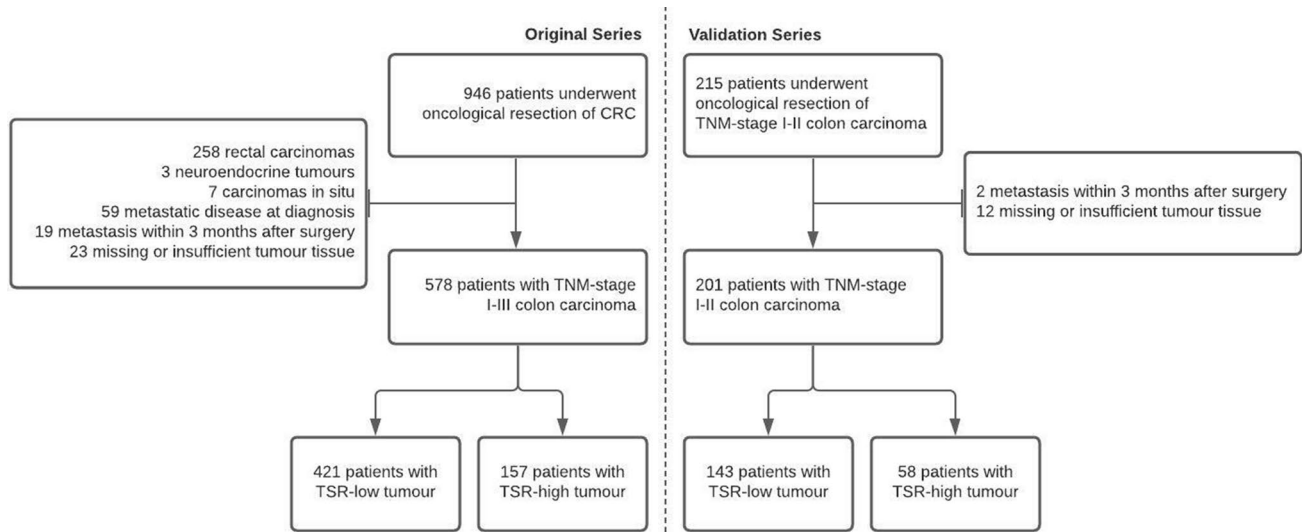
Fig. 1 Flowchart of the study

Data from the Netherlands Cancer Registry (NCR) were used. The NCR collects data on all newly diagnosed cancer patients in the Netherlands, containing patient demographics, tumour characteristics and information on diagnosis and treatment. The tumour-node-metastasis (TNM) classification was used for stage notification of the primary tumour, according to the edition valid at time of cancer diagnosis. Additional data were collected from medical records of the patients. This included information on histopathological characteristics of the tumour, ASA-classification, comorbidities, follow-up and cancer recurrence.

Histopathological material and analysis

The amount of tumour-stroma was estimated by microscopic analysis of 4 μm haematoxylin and eosin (H&E)-stained tissue sections of the primary tumour and the corresponding metastatic lymph nodes using a scoring technique described by van Pelt et al. [24]. In this, an $\times 2.5$ or $\times 5$ objective was used to select the area appearing to have the highest amount of tumour-stroma. Hereafter, an $\times 10$ objective was used to select an area with both tumour-stroma tissue and tumour cells, where tumour cells were present at all quadrants of the selected image field's border. Then, the tumour-stroma ratio (TSR) was estimated in a range from 10 to 90% per 10% increment. A cut-off value of 50% stroma was set to categorize patients as TSR-low ($\leq 50\%$) or TSR-high ($> 50\%$), as determined in earlier research to be most discriminative [20]. In case one metastatic lymph node was TSR-high, the final score on the metastatic lymph nodes of this patient was considered TSR-high. When assessing TSR of both primary tumour and lymph nodes together, TSR was considered high when either the primary tumour or its metastatic lymph nodes was categorized as TSR-high.

Histopathological scoring of TSR was conducted by two independent assessors (MS and AG (research physicians) for the original series and MS and TF (pathology resident) for the validation series) at two different moments segregated by minimum a week time. All assessors gained insight into the scoring technique prior to starting this study by passing a validated e-learning obtained from Leiden University Medical Centre from the UNITED study [25]. Intra- and inter-observer agreement was analysed and consensus between both assessors was reached. In case consensus could not be reached, a third observer (AB, expert pathologist) was decisive.

Endpoints and definitions

Primary endpoints of this study were overall survival (OS) and cancer-free survival (CFS). OS was defined as the time between date of surgery and the date of death or last follow-up (with a maximum of 5 years) in months. CFS was defined as the time from date of surgery until the date of cancer recurrence, defined as the first date of either radiologic or pathologic diagnosis of metastases or tumour recurrence of colorectal cancer, or last follow-up (with a maximum of 5 years) in months. Patients dying without cancer recurrence were censored on the day of death and patients being alive at the date of last follow-up were censored at the date of last follow-up.

Secondary endpoints were inter- and intra-observer agreement in TSR-scores. The intra-observer agreement was defined as the degree of agreement of TSR-scores by one assessor on different moments in time. The inter-observer

agreement was defined as the degree of agreement of TSR-scores between both assessors. Intra- and inter-observer agreement was defined as substantial in case a kappa-coefficient of 0.61–0.80 was reached. In case of a kappa-coefficient above 0.81, the agreement was defined as almost perfect.

Statistical analyses

In this retrospective observational cohort study statistical analyses were conducted for both series separately. Descriptive statistics were performed to provide an overview of both study populations. Continuous variables were expressed as means $\pm$ SD or median with interquartile range when not normally distributed; categorical variables were shown as counts and percentages. Between TSR-groups continuous variables were compared using unpaired t-tests, and categorical variables were compared using Chi-square statistics or Fisher's exact test, as appropriate. Inter- and intra-observer variability were determined using the Cohen kappa coefficient.

Differences in OS and CFS survival according to TSR-groups were visualized by means of Kaplan–Meier curves. Univariable and multivariable cox-regression analyses were conducted to calculate the prognostic association between TSR and OS and CFS, while adjusting for other prognostic variables. For each endpoint, variables included for adjustment were chosen based on clinical judgment, differences at baseline and database availability. Those included patient demographics (age, gender, comorbidities identified at admission according to Charlson Comorbidity Index), tumour characteristics (tumour stage, localisation, differentiation, lymph-angio invasion) and treatment characteristics (surgery, chemotherapy). Goodness of fit of the multivariable models was tested using the 2-log likelihood test. A two-tailed p-value ≤ 0.05 was considered significant in all analyses.

Data was analysed using IBM SPSS Statistics, version 25.0 (IBM Corp, NY, Armonk, USA).

Results

Original series

A total of 578 patients were included with a median age of 71 years (IQR 63–78). All cause-death occurred in 129 patients (22.3%) during a median follow-up of 56 months (IQR 40–60). Cancer recurrence occurred in 95 patients (16.4%) during a median follow-up of 55 months (IQR 37–60).

A total of 157 tumours (27.2%) were classified as TSR-high (> 50%). These tumours were more likely to comprehend one or more of the high-risk features T4 stage, lymph node metastasis and lymph-angio invasion, as described by the ASCO [5], but not poor differentiation (Table 1). Patients with a tumour classified as TSR-high were more likely treated with adjuvant chemotherapy (Table 1). Most patients were treated with both capecitabine and oxaliplatin.

Validation series

The validation series consisted of 201 patients with a median age of 73 years (IQR 65–79). During a median follow-up of 60 months (IQR 35–60), all cause-death occurred in 40 patients (19.9%). Cancer recurrence occurred in 33 patients (16.4%) during a median follow-up of 59 months (IQR 31–81).

In total 58 tumours (28.9%) were classified as TSR-high (> 50%). As in the original series, tumours classified as TSR-high were more likely to comprehend the high-risk feature lymph-angio invasion, as described by the ASCO [5], but not poor differentiation (Table 1). The number T4 stage tumours in this series were too small to comment on differences of this feature between TSR-groups.

Test reproducibility

The degree of agreement in scoring at two different moments by one assessor (intra-observer agreement), and between the final scoring of both assessors (inter-observer agreement) was expressed as Cohen Kappa Coefficient. In the original series this resulted in an intra-observer agreement of 0.798 and 0.738. The inter-observer agreement between MS and AG was stated as 0.781. In the validation series, intra-observer agreements were stated at 0.854 and 0.780, with an inter-observer agreement of 0.876 indicating substantial to almost perfect agreement.

Survival: TNM stage I–II

Original series

All-cause death and cancer recurrence occurred significantly more often in patients with a TSR-high colon carcinoma compared to those with a TSR-low tumour, 23 (28.4%) versus 49 (15.8%), $p = 0.026$, respectively. In the group of patients with a TSR-high colon carcinoma, cancer recurred in significantly more patients compared to the group of patients with a TSR-low tumour, 15 (18.5%) versus 30 (9.6%) $p = 0.009$, respectively. Univariable analysis showed that OS and CFS were significantly lower in patients with TSR-high tumours compared to TSR-low tumours; HR = 1.86 (CI; 1.13–3.06, $p = 0.015$) and HR = 2.04 (CI; 1.10–3.80, $p = 0.024$) for OS and CFS, respectively. After

Table 1 Baseline characteristics of the study population

	Original series			Validation series		
	TSR-low	TSR-high	p-value	TSR-low	TSR-high	p-value
Age*	71 (63—78)	70 (60—77)	0.075	72 (65—80)	73 (65—77)	0.743
Gender, n (%)			0.238			0.864
Male	202 (48.0)	84 (53.5)		77 (53.8)	32 (55.2)	
Female	219 (52.0)	73 (46.5)		66 (46.2)	26 (44.8)	
Comorbidities, n (%)			0.832			0.402
0	212 (50.4)	78 (49.7)		35 (35.7)	15 (38.4)	
1	127 (30.2)	45 (28.7)		31 (31.6)	10 (25.6)	
≥ 2	82 (19.5)	34 (21.7)		32 (32.7)	14 (35.9)	
ASA, n (%)			0.303			
I–II	361 (85.7)	128 (81.5)				
III–IV	49 (11.6)	23 (14.6)				
Missing	11 (2.6)	6 (3.8)				
Surgery, n (%)			0.102			0.103
Elective	399 (94.8)	143 (91.1)		137 (95.8)	51 (87.9)	
Acute	22 (5.2)	14 (8.9)		6 (4.2)	7 (12.1)	
Tumour localisation, n (%)			0.393			0.845
Right colon	172 (40.8)	58 (36.9)		74 (51.7)	31 (53.4)	
Left colon	249 (59.2)	99 (63.1)		66 (46.2)	26 (44.8)	
pT-stage, n (%)			<0.001			—
T1	42 (10.0)	1 (0.6)		4 (2.8)	0 (0.0)	
T2	104 (24.7)	9 (5.7)		18 (12.6)	8 (13.8)	
T3	248 (58.9)	118 (75.2)		121 (84.6)	49 (84.5)	
T4	27 (6.4)	29 (18.4)		0 (0.0)	1 (1.7)	
pN-stage, n (%)			<0.001			
N0	310 (73.6)	79 (50.3)				
N1	80 (19.0)	51 (32.5)				
N2	31 (7.4)	27 (17.2)				
TNM stage, n (%)			<0.001			0.774
I	123 (29.2)	7 (4.5)		22 (15.4)	8 (13.8)	
II	188 (44.7)	74 (47.1)		121 (84.6)	50 (86.2)	
III	110 (26.1)	76 (48.4)				
Differentiation, n (%)			0.015			0.816
Well/moderate	329 (78.1)	139 (88.5)		124 (86.7)	51(87.9)	
Poor/undifferentiated	80 (19.0)	17 (10.8)		19 (13.3)	7 (12.1)	
Missing	12 (2.9)	1 (0.6)				
Lymph-angio invasion, n (%)			0.026			0.023
Yes	60 (14.3)	35 (22.3)		20 (14.0)	16 (27.6)	
No	332 (78.4)	107 (68.2)		123 (86.0)	42 (72.4)	
Missing	29 (6.9)	15 (9.6)				
Chemotherapy, n (%)			<0.001			
Yes	90 (21.4)	66 (42.0)				
No	331 (78.6)	91 (58.0)				
Chemotherapy, n (%)			0.205			
Capecitabine + oxaliplatin	77 (18.3)	49 (31.2)				
Capecitabine monotherapy	11 (2.6)	14 (8.9)				
Other	2 (0.5)	3 (1.9)				

*Non−normal distributed data presented as median with interquartile range

adjustment for some possible confounders (age, tumour stage, differentiation grade and lymph-angio invasion) in multivariable analysis these associations remained significant, HR = 1.71 (CI; 1.02–2.87, $p = 0.042$) and HR = 2.05 (CI; 1.04–4.05, $p = 0.038$).

Validation series

Also in the validation series, cancer recurred more often in patients with a TSR-high tumour compared to those with a TSR-low tumour (14 (24.1%) versus 19 (13.3%), $p = 0.060$), although only a trend that did not reach significance at the $p < 0.05$ level. Both univariable and multivariable analyses (adjusted for age, tumour stage, differentiation grade and lymph-angio invasion) showed that CFS was significantly lower in patients with TSR-high tumours compared to TSR-low tumours, HR = 2.12 (CI; 1.06–4.23, $p = 0.033$) and HR = 2.18 (CI; 1.07–4.43, $p = 0.031$). In this series, there was no significant association between TSR and OS (univariable; HR = 1.34 (CI; 0.69–2.60, $p = 0.383$), multivariable; HR = 1.64 (CI; 0.83–3.25, $p = 0.155$)).

Table 2 shows the associations between TSR and CFS.

Survival: TNM-stage III

TSR-high TNM-stage III tumours were associated with poorer OS and CFS compared to TSR-low TNM-stage III tumours. All-cause death and cancer recurrence occurred in respectively 33 (43.4%) and 29 (38.2%) patients with a TSR-high tumour, compared to 24 (21.8%) and 21 (19.1%) patients with a TSR-low tumour. Univariable analysis showed that OS and CFS were significantly lower in patients with a TSR-high tumour compared to those with a TSR-low tumour, HR = 2.16 (CI; 1.28–3.66, $p = 0.004$) and HR = 2.41 (CI; 1.36–4.27, $p = 0.003$). After adjustment for identified

confounders and other possible risk factors in multivariable analysis, OS and CFS remained significantly poorer in patients with TSR-high tumours, HR = 2.26 (CI; 1.26–4.03, $p = 0.006$) and HR = 2.20 (CI; 1.18–4.08, $p = 0.013$). Lymph-angio invasion and treatment with adjuvant chemotherapy were identified as confounding variables for both OS and CFS. Additional confounding variables included for multivariable analysis were age and N-stage in case of CFS, and age, comorbidities and tumour localisation in case of overall survival.

Lymph node metastases were present in 186 out of 578 patients (original series) according to TNM-classification. However, lymph node slides of 5 patients were missing, leading to exclusion of these patients for TSR assessment of lymph nodes. In 46 patients at least one lymph node was found to be TSR-high. In 58 patients (32.0%) the TSR classification varied between the assessment of the primary tumour and its metastatic lymph nodes (Table 3). Combining TSR-analysis of the primary tumour and lymph nodes led to restaging of 15 patients (8.3%) with a TSR-low tumour to the TSR-high group. This restaging increased the 5-year CFS of the remaining TSR-low group from 80% (TSR-low in PT) to 85% (TSR-low in PT and LN). Within all patients with a TSR-low primary tumour, the risk of cancer recurrence within 5 years after treatment was over two times higher in case of one or more TSR-high lymph nodes ($n = 15$) (unadjusted HR = 2.66 (CI; 1.02–6.93), $p = 0.045$).

Table 3 Overview of TSR on primary tumour versus lymph nodes

Lymph nodes	Primary tumour	
	TSR-low	TSR-high
TSR-low	92 (86.0%)	43 (58.1%)
TSR-high	15 (14.0%)	31 (41.9%)

Table 2 Association between tumour-stroma ratio and cancer-free survival in TNM-stage I-II colon tumours

Original series				Validation series			
		HR (95% CI)	p value			HR (95% CI)	p value
Unadjusted				Unadjusted			
TSR-low ($\leq$ 50%)	($n = 311, 79.3\%$)	1 (reference)		TSR-low ($\leq$ 50%)	($n = 143, 71.1\%$)	1 (reference)	
TSR-high (> 50%)	($n = 81, 20.7\%$)	2.04 (1.10—3.80)	0.028	TSR-high (> 50%)	($n = 58, 28.9\%$)	2.12 (1.06—4.23)	0.033
Model 1				Model 1			
TSR-low ($\leq$ 50%)	($n = 311, 79.3\%$)	1 (reference)		TSR-low ($\leq$ 50%)	($n = 143, 71.1\%$)	1 (reference)	
TSR-high (> 50%)	($n = 81, 20.7\%$)	2.16 (1.14—4.11)	0.018	TSR-high (> 50%)	($n = 58, 28.9\%$)	2.14 (1.07—4.27)	0.031
Model 2				Model 2			
TSR-low ($\leq$ 50%)	($n = 268, 68.4\%$)	1 (reference)		TSR-low ($\leq$ 50%)	($n = 143, 71.1\%$)	1 (reference)	
TSR-high (> 50%)	($n = 66, 16.8\%$)	2.05 (1.04—4.05)	0.038	TSR-high (> 50%)	($n = 58, 28.9\%$)	2.18 (1.07—4.43)	0.031

Model 1. Adjusted for age

Model 2. Adjusted for age, tumour stage, differentiation grade and lymph−angio invasion

HR hazard ratio, *CI* confidence interval, *TSR* tumour−stroma ratio

This TSR-high group (TSR-high in primary tumour and/ or lymph node) showed an even higher HR on cancer recurrence within 5 years after treatment than TSR-high status on the primary tumour alone (HR = 2.14 (CI; 1.12–4.10), $p = 0.022$). Table 4 shows the association between TSR and CFS after restaging according to TSR in metastatic lymph nodes.

Chemo resistance

Out of all 578 patients in the original series, 156 patients received adjuvant chemotherapy (CT) after curative resection of their tumour. Among them were 128 patients (82.1%) with a TNM-stage III tumour, and 28 patients (17.9%) with a TNM-stage II tumour. In another 107 patients CT was indicated according to the current treatment guidelines, but treatment was waived.

Patients receiving CT were significantly younger and had less severe comorbidities, compared to those not receiving adjuvant treatment. The tumour was more often located in the left-sided colon, and surgery was mostly performed in elective setting, It is notable that, when indicated, it was more likely that CT was administered in case of a TNM-stage III tumour, compared to a high-risk TNM-stage II tumour. Table 5 shows the descriptive characteristics of all patients for whom CT was indicated.

The risk of cancer recurrence in patients for whom CT was indicated but treatment was waived did not differ between patients with TSR-low and TSR-high tumours, independent of confounders (HR = 1.10 (CI; 0.44–2.72,

$p = 0.845$)). Patients with TSR-low tumours receiving CT were less likely to experience cancer recurrence compared to those not receiving CT despite the indication for this treatment (HR = 0.34 (CI; 0.14–0.86, $p = 0.023$), independent of the identified confounders (age, comorbidities and tumour stage). In contrast, receiving CT when indicated was not associated with a lower risk of cancer recurrence in TSR-high tumours (HR = 0.90 (CI; 0.40–2.04, $p = 0.799$), independent of identified confounders. Of all patients treated with CT, patients with a TSR-high tumour had a 4.2 times higher risk to develop cancer recurrence compared to patients with a TSR-low tumour, $p < 0.001$. Kaplan–Meier curves for CFS according to adjuvant chemotherapy stratified by TSR were presented in Fig. 2. Table 6 shows the association between CT and cancer recurrence, according to TSR.

Discussion

In this observational retrospective cohort study we validated the tumour-stroma scoring method developed by the Leiden University Medical Centre as a reproducible method with prognostic value. As in other studies, TSR was more often low in TNM-stage I and II disease compared to TNM-stage III disease, which emphasises the importance of stratification on TNM-stage when assessing the prognostic value [26, 27]. In the present study, TSR-high TNM-stage I–II tumours showed to be associated with more than twice the risk of cancer recurrence within 5 years after curative treatment,

Table 4 Association between tumour-stroma ratio and cancer-free survival in TNM-stage III colon tumours

Primary tumour only		HR (95% CI)	p value	Primary tumour and lymph nodes		HR (95% CI)	p value
Unadjusted				Unadjusted			
TSR-low (≤50%)	(n=110, 59.1%)	1 (reference)		PT and LN TSR-low (≤50%)	(n=92, 49.5%)	1 (reference)	
TSR-high (>50%)	(n=76, 40.7%)	2.41 (1.36—4.27)	0.003	PT or/and LN TSR-high (>50%)	(n=89, 47.8%)	2.73 (1.46—5.11)	0.002
Model 1				Model 1			
TSR-low (≤50%)	(n=110, 59.1%)	1 (reference)		PT and LN TSR-low (≤50%)	(n=92, 49.5%)	1 (reference)	
TSR-high (>50%)	(n=76, 40.7%)	2.26 (1.25—4.11)	0.007	PT or/and LN TSR-high (>50%)	(n=89, 47.8%)	2.55 (1.35—4.83)	0.004
Model 2				Model 2			
TSR-low (≤50%)	(n=110, 59.1%)	1 (reference)		PT and LN TSR-Low (≤50%)	(n=92, 49.5%)	1 (reference)	
TSR-high (>50%)	(n=76, 40.7%)	2.07 (1.13—3.77)	0.018	PT or/and LN TSR-High (>50%)	(n=89, 47.8%)	2.14 (1.12—4.10)	0.022

Model 1. Adjusted for age

Model 2. Adjusted for age, N-stage, lymph−angio invasion and treatment with adjuvant chemotherapy (yes/no)

HR hazard ratio, *CI* confidence interval, *TSR* tumour−stroma ratio, *PT* primary tumour, *LN* lymph node

Table 5 Descriptive characteristics of patients for whom adjuvant chemotherapy treatment is indicated

|	No adjuvant chemotherapy	Adjuvant chemotherapy	p value
Age*	77 (69—81)	66 (60—72)	<0.001
Gender, n (%)			0.007
Male	41 (38.3)	86 (55.1)	
Female	66 (61.7)	70 (44.9)	
Comorbidities, n (%)			<0.001
0	37 (34.6)	93 (59.6)	
1	33 (30.8)	46 (29.5)	
≥2	37 (34.6)	17 (10.9)	
ASA, n (%)			<0.001
I–II	73 (68.2)	146 (93.6)	
III–IV	21 (19.6)	10 (6.4)	
Surgery, n (%)			0.001
Elective	84 (78.5)	144 (92.3)	
Acute	24 (21.5)	12 (7.7)	
Tumour localisation, n (%)			0.027
Right colon	53 (49.5)	56 (35.9)	
Left colon	54 (50.5)	100 (64.1)	
pT-stage, n (%)			0.140
T1	1 (0.9)	3 (1.9)	
T2	7 (6.5)	15 (9.6)	
T3	69 (64.5)	112 (71.8)	
T4	30 (28.0)	26 (16.7)	
pN-stage, n (%)			<0.001
N0	48 (44.9)	27 (17.3)	
N1	42 (39.3)	89 (57.1)	
N2	17 (15.9)	40 (25.6)	
TNM stage, n (%)			<0.001
II	49 (45.8)	28 (17.9)	
III	58 (54.2)	128 (82.1)	
Differentiation, n (%)			0.838
Well/moderate	82 (76.6)	120 (76.9)	
Poor/undifferentiated	24 (22.4)	33 (21.2)	
Lymph-angio invasion, n (%)			0.903
Yes	37 (34.6)	54 (34.6)	
No	63 (58.9)	95 (60.9)	
Chemotherapy, n (%)			
Capecitabine + oxaliplatin		126 (80.8)	
Capecitabine monotherapy		25 (16.0)	
Other		5 (3.2)	

*Non−normal distributed data presented as median with interquartile range

in two independent series, independent of most known high-risk features [5]. In the original series, this reflected on the OS, with significantly poorer survival in TSR-high tumours. This was not confirmed for the validation series, in which more patients probably died due to non-cancer related causes. However, the prognostic value for cancer recurrence, also found in several other studies, implies that TSR is a new high-risk feature that should help us improve the identification of lymph node negative high-risk cases [19–22].

In lymph node positive patients, TSR of the primary tumour showed to be of prognostic value for CFS, independent of most known high-risk features and treatment with adjuvant chemotherapy. Additionally, we have shown that combining TSR of the primary tumour and metastatic lymph nodes adds prognostic value to TSR of the primary

Fig. 2 Kaplan–Meier curves of cancer-free survival according to **a** adjuvant chemotherapy in TSR-low tumours, **b** adjuvant chemotherapy in TSR-high tumours
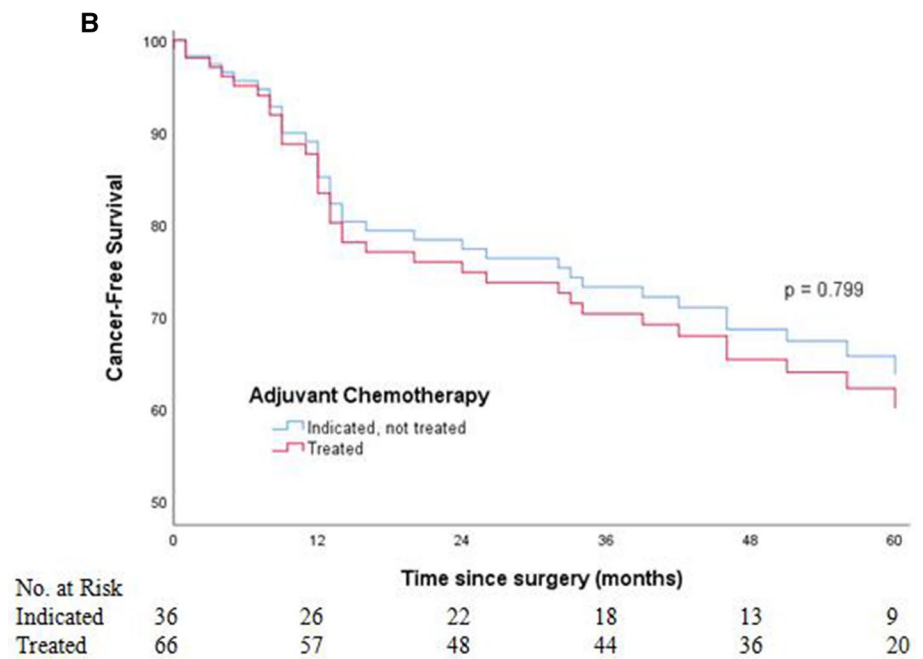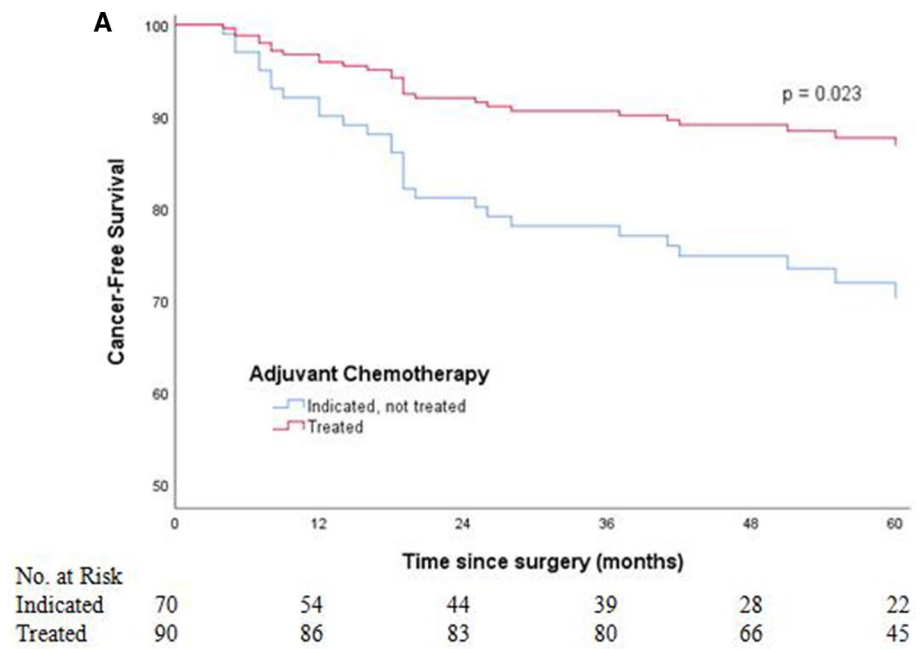
tumour alone, regardless of N-stage. This result is in line with the findings of the only other study examining TSR in metastatic lymph nodes ($n = 102$) in colon cancer [22]. TSR in the primary tumour and its metastatic lymph nodes was heterogeneous, which may be explained by the changing intracellular signalling within the tumour microenvironment during tumour progression [28]. Such heterogeneity has also been described by studies investigating expression patterns of other prognostic markers [29–31]. From this, we believe that TSR has an additional value in predicting CFS in TNM-stage III colon cancer, and should from now on be assessed in both primary tumour and metastatic lymph nodes.

While TSR proves to be of prognostic value in oncological outcome and can aid in the identification of patients at high-risk of cancer recurrence, our results do not support treatment with adjuvant chemotherapy in these by TSR defined high-risk patients. No improvement of CFS in patients with a TSR-high tumour receiving adjuvant

Table 6 Association between adjuvant treatment with chemotherapy and cancer-free survival, stratified according to TSP-groups

TSR-low		HR (95% CI)	p value	tsr-high		HR (95% CI)	p value
Unadjusted				Unadjusted			
No CT	(n = 71, 66.4%)	1 (reference)		No CT	(n = 36, 33.6%)	1 (reference)	
CT	(n = 90, 57.7%)	0.37 (0.17—0.79)	0.010	CT	(n = 66, 42.3%)	1.15 (0.55—2.40)	0.708
Model 1				Model 1			
No CT	(n = 71, 66.4%)	1 (reference)		No CT	(n = 36, 33.6%)	1 (reference)	
CT	(n = 90, 57.7%)	0.41 (0.17—0.98)	0.045	CT	(n = 66, 42.3%)	1.14 (0.53—2.48)	0.737
Model 2				Model 2			
No CT	(n = 71, 66.4%)	1 (reference)		No CT	(n = 36, 33.6%)	1 (reference)	
CT	(n = 90, 57.7%)	0.34 (0.14—0.86)	0.023	CT	(n = 66, 42.3%)	0.90 (0.40—2.04)	0.799

Model 1. Adjusted for age and comorbidities

Model 2. Adjusted for age, comorbidities, and tumour stage

HR hazard ratio, *CI* confidence interval, *TSR* tumour−stroma ratio, *CT* chemotherapy

chemotherapy was found, while adjuvant chemotherapy led to significant improvement of CFS in patients with a TSR-low tumour. These results are in line with results found in studies regarding the value of TSR as predictor of response to neoadjuvant chemoradiotherapy [32, 33]. So, not only tumour invasion and metastasis, but also treatment response is largely influenced by the tumour microenvironment, a theory supported by the results of our study [34]. The tumour microenvironment is characterised by rigidity, hypoxia and an altered composition of paracrine factors [35]. Hypoxia leads to a reduction in drug availability through upregulation of drug transporters promoting drug efflux, decreasing the catalytic ability of cytochrome p450 which leads to drug retention, and creating an acid environment reducing the drugs ability to cross the hydrophobic plasma membrane of cancer cells. Matrix rigidity activated signalling pathways associated with cell survival and apoptosis, while paracrine factors of the tumour microenvironment promote pro-survival signalling pathways promoting cancer stem cell self-renewal [35–39]. All three characteristics promote EMT, which is known to facilitate cell migration, aid invasiveness and increases resistance to apoptosis, and to be highly chemo resistant [35–39]. Moreover, in vitro models show that the tumour microenvironment not only causes chemo resistance, but also promotes further tumour growth by upregulation of growth factors as TGF-β and IL-17A when targeted by chemotherapy [34]. This stretches the importance of identifying treatments targeting tumour microenvironment in addition to targeting tumour cells alone.

The present study strengthens the value of TSR as prognostic marker in the identification of patients at high-risk of cancer recurrence in both lymph node positive and negative patients, regardless of other known high-risk features. It also confirms the additional prognostic value of TSR in metastatic lymph nodes to TSR in the primary tumour alone in lymph node positive patients. Not only can TSR serve as a prognostic marker that aids the identification of patients at high-risk of cancer recurrence, but also of those less likely to benefit from standard adjuvant chemotherapy. This implicates its potential role in a therapeutic algorithm needed to ensure that the risk of treatment-related complications is only taken when benefit of treatment is likely. When implementing TSR as additional prognostic marker, treatment risks can be further specified, which is especially important in this era of shared decision making and an aging population bringing more age-related risk factors for developing treatment-related complications. Benefits of other treatments, targeting stromal components as immune cells and extracellular matrix components, should be further explored as it is believed that combination therapies targeting both cancer- and stromal cells can generate better tumour response [40].

The main limitation of this study was its retrospective character. For this, selection bias could not be fully excluded due to missing tumour slides and the use of a validation series which was formed prior to this study, lacking TNM-stage III tumours and possibly excluding some TNM-stage II patients receiving chemotherapy. Also, residual confounding may have occurred despite the fact that we were able to adjust for some important confounders within database availability. Unfortunately, we were not able to determine cancer-specific survival due to missing information about cause of death in a large number of patients. There were also some important strengths to this study. The large number of included patients allowed us to stratify according to lymph node positive and lymph node negative disease, and conduct sub-analyses for patients for whom adjuvant chemotherapy was indicated, while correcting for multiple known confounders. The entirety of variables in this dataset enabled us to include most possible confounders.

In conclusion, the TSR in colon cancer in both primary tumour and lymph node metastasis adds significant prognostic value to current pathologic and clinical features used for the identification of patients at high risk of cancer recurrence. A high TSR is not only associated with a poor CFS in TNM-stage I–III colon cancer, but also predicts chemo resistance in TNM-stage II–III colon cancer, which stretches the importance of identifying treatments targeting the tumour microenvironment to improve the prognosis of these high-risk patients by giving them a suitable treatment. A prospective study validating TSR as a prognostic marker would be preferable, and has already been started [41] . However, since the prognostic value of TSR has now been described in several retrospective studies, and TSR scoring shows to be easy, highly reproducible and low cost, we recommend incorporating TSR in the current list of high-risk features.

Data availability Data and material cannot be shared publicly because of ethical concerns. Patients were included on a no objection base to conduct retrospective data studies and publish findings, but were not asked for permission to publish full encrypted data. Data are available from the VieCuri Institutional Data Access (contact via wetenschapsbureau@viecuri.nl) for researchers who meet the criteria for access to confidential data.

Code availability Codes are available upon request via corresponding author.

Declarations

Conflict of interest The authors have declared that no competing interest exists.

Ethical approval (Research involving human participants and/or animals) This study was approved by the research committee and the Board of Directors of VieCuri Medical Centre and Jeroen Bosch Hospital. Data were obtained under the law 'scientific research and statistics in the interest of public health, where asking for permission is not possible or appropriate for several reasons' in the Netherlands, unless patients objected to use of their personal medical record for scientific research. Data were encrypted with an encryption key provided by the NCR. Encryption was shortly lifted to access the patients' number for accessing his/her medical record. Following extraction data were encrypted again. Prior collected material was reassessed. A waiver of informed consent was given by the METC Maastricht under the reference number 2020-1336.

Informed consent Data were obtained under the law 'scientific research and statistics in the interest of public health, where asking for permission is not possible or appropriate for several reasons' in the Netherlands, unless patients objected to use of their personal medical record for scientific research.

References

1. Dutch guideline 'colonic cancer' 2019. https://richtlijnendatabase.nl/richtlijn/colorectaal_carcinoom_crc/primaire_behandeling_coloncarcinoom_bij_crc.html.
2. van Pelt GW, Sandberg TP, Morreau H, Gelderblom H, van Krieken J, Tollenaar R, et al. The tumour-stroma ratio in colon cancer: the biological role and its prognostic impact. Histopathology. 2018;73(2):197–206.
3. Hyslop T, Waldman SA. Molecular staging of node negative patients with colorectal cancer. J Cancer. 2013;4(3):193–9.
4. O'Connell JB, Maggard MA, Ko CY. Colon cancer survival rates with the new American Joint Committee on Cancer sixth edition staging. J Natl Cancer Inst. 2004;96(19):1420–5.
5. Benson AB 3rd, Schrag D, Somerfield MR, Cohen AM, Figueredo AT, Flynn PJ, et al. American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol. 2004;22(16):3408–19.
6. O'Connor ES, Greenblatt DY, LoConte NK, Gangnon RE, Liou JI, Heise CP, et al. Adjuvant chemotherapy for stage II colon cancer with poor prognostic features. J Clin Oncol. 2011;29(25):3381–8.
7. Conti J, Thomas G. The role of tumour stroma in colorectal cancer invasion and metastasis. Cancers (Basel). 2011;3(2):2160–8.
8. De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. Int J Cancer. 2008;123(10):2229–38.
9. Calon A, Espinet E, Palomo-Ponce S, Tauriello DV, Iglesias M, Cespedes MV, et al. Dependency of colorectal cancer on a TGF-beta-driven program in stromal cells for metastasis initiation. Cancer Cell. 2012;22(5):571–84.
10. Hawinkels LJ, Paauwe M, Verspaget HW, Wiercinska E, van der Zon JM, van der Ploeg K, et al. Interaction with colon cancer cells hyperactivates TGF-beta signaling in cancer-associated fibroblasts. Oncogene. 2014;33(1):97–107.
11. Siegel PM, Massague J. Cytostatic and apoptotic actions of TGF-beta in homeostasis and cancer. Nat Rev Cancer. 2003;3(11):807–21.
12. Vermeulen L, De Sousa EMF, van der Heijden M, Cameron K, de Jong JH, Borovski T, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nat Cell Biol. 2010;12(5):468–76.
13. Martinez-Outschoorn UE, Lisanti MP, Sotgia F. Catabolic cancer-associated fibroblasts transfer energy and biomass to anabolic cancer cells, fueling tumor growth. Semin Cancer Biol. 2014;25:47–60.
14. Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, et al. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. Science. 2010;330(6005):827–30.
15. Sandberg TP, Stuart M, Oosting J, Tollenaar R, Sier CFM, Mesker WE. Increased expression of cancer-associated fibroblast markers at the invasive front and its association with tumor-stroma ratio in colorectal cancer. BMC Cancer. 2019;19(1):284.
16. Park JE, Lenter MC, Zimmermann RN, Garin-Chesa P, Old LJ, Rettig WJ. Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. J Biol Chem. 1999;274(51):36505–12.

17. Denko NC. Hypoxia, HIF1 and glucose metabolism in the solid tumour. Nat Rev Cancer. 2008;8(9):705–13.

18. Hale MD, Hayden JD, Grabsch HI. Tumour-microenvironment interactions: role of tumour stroma and proteins produced by cancer-associated fibroblasts in chemotherapy response. Cell Oncol (Dordr). 2013;36(2):95–112.

19. Mesker WE, Junggeburt JM, Szuhai K, de Heer P, Morreau H, Tanke HJ, et al. The carcinoma-stromal ratio of colon carcinoma is an independent factor for survival compared to lymph node status and tumor stage. Cell Oncol. 2007;29(5):387–98.

20. Park JH, Richards CH, McMillan DC, Horgan PG, Roxburgh CSD. The relationship between tumour stroma percentage, the tumour microenvironment and survival in patients with primary operable colorectal cancer. Ann Oncol. 2014;25(3):644–51.

21. Huijbers A, Tollenaar RA, v Pelt GW, Zeestraten EC, Dutton S, McConkey CC, et al. The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. Ann Oncol. 2013;24(1):179–85.

22. van Pelt GW, Hansen TF, Bastiaannet E, Kjaer-Frifeldt S, van Krieken JHJ, Tollenaar RA, et al. Stroma-high lymph node involvement predicts poor survival more accurately for patients with stage III colon cancer. J Med Surg Pathol. 2016;1:1–8.

23. Vangangelt KMH, Green AR, Heemskerk IMF, Cohen D, van Pelt GW, Sobral-Leite M, et al. The prognostic value of the tumor-stroma ratio is most discriminative in patients with grade III or triple-negative breast cancer. Int J Cancer. 2020;146(8):2296–304.

24. van Pelt GW, Kjaer-Frifeldt S, van Krieken J, Al Dieri R, Morreau H, Tollenaar R, et al. Scoring the tumor-stroma ratio in colon cancer: procedure and recommendations. Virchows Arch. 2018;473(4):405–12.

25. Smit MA, van Pelt GW, Dequeker EM, Al Dieri R, Tollenaar RA, van Krieken JHJ, et al. e-Learning for instruction and to improve reproducibility of scoring tumor-stroma ratio in colon carcinoma: performance and reproducibility assessment in the UNITED study. JMIR Form Res. 2021;5(3):e19408.

26. Eriksen AC, Sorensen FB, Lindebjerg J, Hager H, de Pont Christensen R, Kjaer-Frifeldt S, et al. The prognostic value of tumour stroma ratio and tumour budding in stage II colon cancer. A nationwide population-based study. Int J Colorectal Dis. 2018;33(8):1115–24.

27. Hutchins GGA, Treanor D, Wright A, Handley K, Magill L, Tinkler-Hundal E, et al. Intratumoral stromal morphometry predicts disease recurrence but not response to 5-fluorouracil-results from the QUASAR trial of colorectal cancer. Histopathology. 2018;72(3):391–404.

28. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.

29. McKay JA, Douglas JJ, Ross VG, Curran S, Ahmed FY, Loane JF, et al. Expression of cell cycle control proteins in primary colorectal tumors does not always predict expression in lymph node metastases. Clin Cancer Res. 2000;6(3):1113–8.

30. McKay JA, Murray LJ, Curran S, Ross VG, Clark C, Murray GI, et al. Evaluation of the epidermal growth factor receptor (EGFR) in colorectal tumours and lymph node metastases. Eur J Cancer. 2002;38(17):2258–64.

31. Zalata KR, Elshal MF, Foda AA, Shoma A. Genetic dissimilarity between primary colorectal carcinomas and their lymph node metastases: ploidy, p53, bcl-2, and c-myc expression—a pilot study. Tumour Biol. 2015;36(8):6579–84.

32. Hagenaars SC, de Groot S, Cohen D, Dekker TJA, Charehbili A, Meershoek-Klein Kranenbarg E, et al. Tumor-stroma ratio is associated with Miller-Payne score and pathological response to neoadjuvant chemotherapy in HER2-negative early breast cancer. Int J Cancer. 2021.

33. van Pelt GW, Krol JA, Lips IM, Peters FP, van Klaveren D, Boonstra JJ, et al. The value of tumor-stroma ratio as predictor of pathologic response after neoadjuvant chemoradiotherapy in esophageal cancer. Clin Transl Radiat Oncol. 2020;20:39–44.

34. Lotti F, Jarrar AM, Pai RK, Hitomi M, Lathia J, Mace A, et al. Chemotherapy activates cancer-associated fibroblasts to maintain colorectal cancer-initiating cells by IL-17A. J Exp Med. 2013;210(13):2851–72.

35. Yeldag G, Rice A, Del Rio Hernandez A. Chemoresistance and the self-maintaining tumor microenvironment. Cancers (Basel). 2018;10(12).

36. Comerford KM, Wallace TJ, Karhausen J, Louis NA, Montalto MC, Colgan SP. Hypoxia-inducible factor-1-dependent regulation of the multidrug resistance (MDR1) gene. Cancer Res. 2002;62(12):3387–94.

37. Lee KA, Roth RA, LaPres JJ. Hypoxia, drug therapy and toxicity. Pharmacol Ther. 2007;113(2):229–46.

38. Sriraman SK, Aryasomayajula B, Torchilin VP. Barriers to drug delivery in solid tumors. Tissue Barriers. 2014;2:e29528.

39. Zhao J. Cancer stem cells and chemoresistance: The smartest survives the raid. Pharmacol Ther. 2016;160:145–58.

40. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. Nat Rev Clin Oncol. 2018;15(6):366–81.

41. UNITED Study. http://watchstroma.com.