Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma

Scheer, Rene; Baidoshvili, Alexi; Zoidze, Shorena; Elferink, Marloes A. G.; Berkel, Annefleur E. M.; Klaase, Joost M.; van Diest, Paul J.

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### MINIREVIEWS

457  Neoadjuvant therapy for resectable pancreatic cancer  
Rahman SH, Urquhart R, Molinari M

### ORIGINAL ARTICLE

**Retrospective Cohort Study**

466  Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma: A retrospective cohort study  
Scheer R, Baidoshvili A, Zoidze S, Elferink MAG, Berkel AEM, Klaase JM, van Diest PJ

### META-ANALYSIS

475  Laparoscopic vs open complete mesocolic excision with central vascular ligation for colon cancer: A systematic review and meta-analysis  
Negoi I, Hostiuc S, Negoi RI, Beuran M

### CASE REPORT

492  Cystic metastasis from a mucinous adenocarcinoma of duodenum mimicking type II choledochal cyst: A case report  
Kim YN, Song JS

497  Extrapancreatic solid pseudopapillary neoplasm followed by multiple metastases: Case report  
Wu H, Huang YF, Liu XH, Xu MH
World Journal of Gastrointestinal Oncology
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Tumor-stroma ratio as prognostic factor for survival in rectal adenocarcinoma: A retrospective cohort study

René Scheer, Alexi Baidoshvili, Shorena Zoidze, Marloes AG Elferink, Annefleur EM Berkel, Joost M Klaase, Paul J van Diest

AIM
To evaluate the prognostic value of the tumor-stroma ratio (TSR) in rectal cancer.

METHODS
TSR was determined on hematoxylin and eosin stained histological sections of 154 patients treated for rectal adenocarcinoma without prior neoadjuvant treatment in the period 1996-2006 by two observers to assess presented data are anonymized and risk of identification is low.

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Abstract

AIM
To evaluate the prognostic value of the tumor-stroma ratio (TSR) in rectal cancer.

METHODS
TSR was determined on hematoxylin and eosin stained histological sections of 154 patients treated for rectal adenocarcinoma without prior neoadjuvant treatment in the period 1996-2006 by two observers to assess
reproducibility. Patients were categorized into three categories: TSR-high [carcinoma percentage (CP) \(\geq 70\)%], TSR-intermediate (CP 40%, 50% and 60%) and TSR-low (CP \(\leq 30\)%). The relation between categorized TSR and survival was analyzed using Cox proportional hazards model.

RESULTS
Thirty-six (23.4%) patients were scored as TSR-low, 70 (45.4%) as TSR-intermediate and 48 (31.2%) as TSR-high. TSR had a good interobserver agreement (\(\kappa = 0.724\), concordance 82.5%). Overall survival (OS) and disease free survival (DFS) were significantly better for patients with a high TSR (\(P = 0.01\) and \(P = 0.02\), respectively). A similar association existed for disease specific survival (\(P = 0.06\)). In multivariate analysis, patients without lymph node metastasis and an intermediate TSR had a higher risk of dying from rectal cancer (HR = 5.27, 95%CI: 1.54-18.10), compared to lymph node metastasis negative patients with a high TSR. This group also had a worse DFS (HR = 6.41, 95%CI: 1.84-22.28). An identical association was seen for OS. These relations were not seen in lymph node metastasis positive patients.

CONCLUSION
The TSR has potential as a prognostic factor for survival in surgically treated rectal cancer patients, especially in lymph node negative cases.

Key words: Rectal cancer; Adenocarcinoma; Prognosis; Recurrence; Pathology; Tumor-stroma ratio

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Core tip: The tumor-stroma ratio (TSR) can be determined accurately on routine histopathological sections by different observers. The TSR has potential as a prognostic factor for survival in surgically treated rectal cancer patients, especially in lymph node negative cases. It could therefore be useful in decision making regarding adjuvant treatment in individual patients.

INTRODUCTION
Colorectal cancer (CRC) is a common form of cancer in both men and women. More than 15000 new patients with a colorectal carcinoma were diagnosed in The Netherlands in 2016[1]. The common form to stage this type of cancer is the TNM staging system of the Union Internationale Contre le Cancer/American Joint Cancer Committee (UICC/AJCC)[2]. This system is also used in decision making about the appliance of (neo)adjuvant (chemo)radiotherapy. Although the TNM staging system is still regarded as the most important prognostic factor[3], it seems insufficiently able to predict the prognosis of the individual patient. This applies in particular to patients with stage II rectal cancer[4]. A part of the patients is overtreated and consequently exposed to a higher risk on therapy related complications, indicating a need for additional prognostic factors.

More recently, some studies have focused on the tumor-host interaction in relation to metastatic invasion. This interaction is enacted in an environment including cancer cells, the stromal tissue, consisting of different cell types like fibroblasts, myofibroblasts, endothelial cells and immune cells, and the extracellular matrix[5]. Mesker et al[5] showed that a high tumor-stroma ratio (TSR), the proportion of carcinoma relative to the proportion of tumor stroma in the histopathological section through the tumor, is an indicator of a better outcome of disease in colon cancer. This is more outspoken for right sided tumors[5]. Similar results were seen in breast cancer, oral squamous cell carcinoma and prostate cancer[7-9]. A high TSR is possibly related to both a longer disease free and overall survival (OS) according to a study on a small number of rectal cancer patients[10]. In this respect, it is meaningful to explore the relevance of the TSR in a larger cohort of patients with rectal adenocarcinoma.
fashion according to the Code for proper secondary use of human tissue from the Dutch Federation of Medical Scientific Societies and with respect to the Helsinki Declaration.

**TSR assessment**

TSR was determined on hematoxylin and eosin (H and E) stained histological sections. The section with the most invasive part of the tumor was identified to semiquantitatively assess the carcinoma percentage (CP) in 10% steps. The CP is a derivative of the TSR and is complementary to the percentage of stroma and other components, like mucus. For example, a CP of 20% corresponds to a stroma percentage of 80%, which coincides with a low TSR. The section was viewed with a 5 × objective (50 times magnification). The CPs were determined on all image fields of the entire section with tumor cells in all sides of it (North-East-South-West). Areas with the lowest CP were given more weight in rating the mean CP of the total assessed area, as is common practice in routine pathology in determining tumor differentiation. All sections were assessed separately by two observers (René Scheer and Shorena Zoidze) to allow assessment of reproducibility. The absolute CPs were categorized for a good clinical reproducibility and clarity reasons into three categories, finally. TSR-low including the CP-values \( \leq 30\% \), TSR-intermediate including the CP-values 40%, 50% and 60%, and TSR-high including the CP-values \( \geq 70\% \) (Figure 2). In the results only the categorized TSR are shown for clarity.

In case of a difference of 10% in determined CPs, which lead to a different TSR category, the lowest CP was used for the determination of the final TSR. The sections were reviewed by a third observer (AB) in case of > 10% difference in determined CPs leading
to different TSR categories. This third opinion was considered as decisive.

**Statistical analysis**

Data were analyzed using SPSS, version 19.0 (SPSS Inc., Chicago, IL, United States) and Stata, version 12.0 (StataCorp LP, College Station, Texas, United States). The statistical methods of this study were reviewed by Elferink MA, from the Netherlands Comprehensive Cancer Organization.

Patient characteristics were compared using Pearson χ² tests and one-way ANOVA. Interobserver reproducibility for the absolute and categorized CPs was analyzed by using Cohen’s Kappa (κ) coefficient. A κ-value of 0.0 or less was considered to represent poor agreement, 0.01-0.20 slight agreement, 0.21-0.40 fair, 0.41-0.60 moderate, 0.61-0.80 sufficient to good, and 0.81-1.00 near-perfect agreement. Survival analyses based on categorized CPs included comparison of OS, disease-free survival (DFS), and disease-specific survival (DSS) by Kaplan-Meier survival analysis and log-rank statistics. Follow-up time in OS analyses was defined as the period between the date of primary surgery and the date of death from any cause, or the date of last follow-up. The DSS was restricted to death from rectal cancer only. Follow-up time in DFS analyses was defined as the time from the date of primary surgery until the date of a local recurrence or distant metastasis (irrespective of site). In DFS analyses, cases were censored in case of a second primary tumor (colorectal or other types) or death. The date of last follow-up was used as endpoint to calculate follow-up time, if none of these events occurred.

The relation between categorized TSR and survival (OS, DFS, and DSS) was analyzed, and adjusted for confounders (age, gender, grading, pathological T- and N-stage, and adjuvant treatment), using Cox proportional hazards model. Probability values < 0.05 (2-sided) were considered statistically significant.

**RESULTS**

**Patient characteristics**

A total of 154 patients met the inclusion criteria for this study. Three types of resections were used: Abdominoperineal resection in 67 (43.5%), low anterior resection in 63 (40.9%), and Hartmann resection, a modulated low anterior resection without construction of an anastomosis, in 24 patients (15.6%). The median follow-up of all patients was 5.3 years. Out of the analyzed samples, 36 (23.4%) were scored as TSR-low, 70 (45.4%) as TSR-intermediate, and 48 (31.2%) as TSR-high. There were more lymph node metastasis positive patients with a low TSR in comparison with patients with a higher TSR \( (P = 0.029) \), who consequently received adjuvant treatment. Radiotherapy was the most common form of adjuvant therapy. Detailed patient characteristics are shown by categorized TSR in Table 1.

Figure 2  Examples of different categories of the tumor-stroma ratio. H and E stained 2 µm paraffin sections of primary rectal adenocarcinoma. TSR-high (carcinoma percentage \( \geq 70\% \)), TSR-intermediate (carcinoma percentage 40%, 50% and 60%), and TSR-low (carcinoma percentage \( \leq 30\% \)). TSR: Tumor-stroma ratio.
Interobserver reproducibility
A third opinion about a final TSR in case of inter-observer disagreement about CPs with > 10% difference in determined CPs leading to different TSR categories was needed in 12 patients (7.8%). Mainly strong heterogeneity of the tumor complicated the determination of the CP for the total section. CPs were scored in a range between 10 and 90 percent. Lower CPs were found in mucinous adenocarcinomas.

Cohen’s Kappa (κ) coefficient for interobserver agreement of the absolute CP showed a moderate agreement (κ = 0.522, concordance 59.1%). By categorizing the CP into three categories TSR (TSR-low, TSR-intermediate, and TSR-high) the κ-value improved and showed a good agreement (κ = 0.724, concordance 82.5%).

Prognostic impact on outcome
The 5-year survival rate for OS was 64.6% in the TSR-high population, vs 50.0% and 55.6% in the TSR-intermediate and TSR-low population, respectively. For the DFS, the 5-year survival rates for TSR-high, TSR-intermediate, and TSR-low were 77.2%, 51.8%, and 55.2%, respectively. The OS and DFS were significant different between the three TSR categories (P = 0.01 and P = 0.02, respectively). The 5-year survival rates for DSS were 81.6% for TSR-high, 60.3% for TSR-intermediate, and 63.9% for TSR-low. Although a higher DSS for the TSR-high population was thereby seen, the differences between the three TSR categories were just not significant (P = 0.06). The Kaplan-Meier survival curves are shown in Figure 3.

After adjusting for known prognostic factors (age, grading, and the use of adjuvant therapy), an intermediate TSR in lymph node metastasis negative patients showed a trend to a lower OS rate (HR = 2.04, 95%CI: 0.99-4.21) in comparison with a high TSR. There were no statistical differences between the TSR categories in OS among lymph node metastasis positive patients (Table 2). A statistically significant worse DFS was seen among the lymph node metastasis negative patients with an intermediate TSR (HR = 6.41, 95%CI: 1.84-22.28) compared to patients with a high TSR. Among lymph node metastasis positive patients, no statistically significant differences were seen between TSR categories for DFS (Table 3). Lymph node meta-

**Table 1  Patient characteristics by categorized tumor-stroma ratio**

| Gender | TSR-low (n = 36) | TSR-intermediate (n = 70) | TSR-high (n = 48) | P-value
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<td>(range 49.0-82.0)</td>
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1Pearson χ² test; 2One-Way ANOVA; 3P-value for adjuvant treatment in general. Significant P-values are shown bold. n: Number of patients; %: Percentage; Age defined as period from birth until date of primary surgery; LAR: Low anterior resection; APR: Abdominoperineal resection; pT: Pathological tumor status; pN: Pathological nodal status; Stage according to UICC/AJCC TNM staging system, 5th edition; TSR: Tumor-stroma ratio; UICC/AJCC: Union Internationale Contre le Cancer / American Joint Cancer Committee.

Scheer R et al. Tumor-stroma ratio in rectal cancer
stasis negative patients with an intermediate TSR had a higher risk of dying from rectal cancer (HR 5.27, 95%CI: 1.54-18.10) in comparison with patients with a high TSR. These differences were not seen in lymph node metastasis positive patients (Table 4).

**DISCUSSION**

This study, analyzing data of 154 patients with rectal adenocarcinoma diagnosed in the period 1996-2006, showed that the TSR is a prognostic factor for patients without lymph node metastasis. In such cases, a high TSR had a longer local recurrence and distant metastasis free period, and a lower risk of death from rectal adenocarcinoma. Besides, a high TSR was associated with a lower risk of death from any cause. The determination of the TSR may therefore contribute to stratify patients for prognosis. Determination of the TSR turned out to be feasible and reproducible among observers on routinely made sections of rectal cancers. The TSR therefore has the potential to contribute to decision making regarding the individual treatment policy in rectal cancer.

The relation between the prognosis and the TSR may be explained pathophysiologically. A dual effect of the tumor stroma in the tumor-host interaction has been described. The tumor stroma is able to exert inhibitory effects on the malignant cells at first. With ongoing tumor growth, the tumor can exploit its stroma, for example by changing its composition (e.g., vasculature), to promote tumor growth and metastasis. A process called stromagenesis, which occurs parallel with tumor progression. Stromagenesis is characterized by bidirectional communication between the tumor and its stroma. The interactional pathways are multiple and complex\[^{[12-14]}\]. Despite this complexity it is justifiable to conclude that the stromal tissue is not a passive component surrounding the tumor. A sufficient amount of stroma contributes to a more aggressive phenotype of tumor, as is shown in this study as well.

Indeed, the poor prognosis for lymph node negative patients with an intermediate TSR is remarkable. The survival rates for death from all causes, death from rectal cancer, and the occurrence of local recurrences and distant metastasis are the lowest for this group of patients. This may be explained by a favorable balance between the tumor and its stroma. In this way, the tumor may be able to exploit the surrounding tumor stroma very efficiently. The concept of a balance between pro- and antitumor factors had been hypothesized earlier. For example, there is a relation between the degree of the peritumoral inflammatory

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Scheer R et al. Tumor-stroma ratio in rectal cancer

**Figure 3** Kaplan-Meier survival curves of overall survival, disease free survival and disease specific survival by categorized tumor-stroma ratio. A: Overall survival; B: Disease free survival; C: Disease specific survival. P-values of Log-rank statistics. TSR-high (carcinoma percentage ≥ 70%), TSR-intermediate (carcinoma percentage 40%, 50% and 60%), and TSR-low (carcinoma percentage ≤ 30%). TSR: Tumor-stroma ratio.
The effect of a high TSR on survival demonstrated in this study has some shortcomings to be noted. The interobserver agreement for absolute scores may be attributed to the determination of other malignancies\(^{[6-10,16]}\). The present study is however the first that has identified a subgroup of patients with rectal cancer, namely lymph node metastasis negative patients with an intermediate TSR, whereby the TSR is a strong prognostic factor.

The interobserver agreement for absolute scores was moderate. The correlation coefficient improved to good, when grouping as TSR-low, TSR-intermediate, and TSR-high. The categorization into these categories was performed with the aim of generating enhanced prognostic information based on the TSR, which had been executed earlier in a previous study on the TSR in oesophageal adenocarcinomas\(^{[17]}\). Other studies concerning the TSR used an arbitrary cut-off value of 50%. No differences in the given survival rates were found at this and other cut-off values in our population (Appendix 1). The rate of agreement of the present study is slightly lower compared to these studies\(^{[7,8]}\), which may be attributed to the determination of absolute CPs before the categorization and the addition of an extra CP-category.

This study has some shortcomings to be noted. Neo-adjuvant radiotherapy for rectal malignancies was applied more frequently at our hospital relatively late in the study period and consequently patients received adjuvant therapy frequently. Neoadjuvant treated patients were excluded, while in most cases a neoadjuvant (chemo)radiotherapy regimen is given nowadays. Though, it remains valuable to investigate tissue based prognostic factors in non-pretreated patients. There is a tendency to treat elderly, for whom there is an increasing incidence of rectal cancer, without neoadjuvant radiotherapy due to postoperative reaction and its ability to destroy invading colorectal malignant cells\(^{[15]}\). Lymph node metastasis can be seen as an expression of a developed tumor that has exploited its environment successfully. When lymph node metastasis has occurred, the effect of the tumor micro-environment may be negligible. This statement may explain why we did not find differences in survival in lymph node metastasis positive patients survival based on the TSR.

The effect of a high TSR on survival demonstrated in this study is in line with other studies on the prognostic impact of the TSR in colorectal carcinomas (CRCs) and

Table 2 Cox multivariate analysis for overall survival

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\(^{*}P < 0.05\); \(^{1}\) Too small numbers to analyze. Age defined as period from birth until date of primary surgery.

N0: Lymph node metastasis negative patients; N+: Lymph node metastasis positive patients; pT: Pathological tumor status; TSR: Tumor-stroma ratio.

Table 3 Cox multivariate analysis for disease free survival

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<th></th>
<th>N0</th>
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\(^{*}P < 0.05\); \(^{1}\) Too small numbers to analyze. Age defined as period from birth until date of primary surgery.

N0: Lymph node metastasis negative patients; N+: Lymph node metastasis positive patients; pT: Pathological tumor status; TSR: Tumor-stroma ratio.
wound complications in The Netherlands. According to the Dutch Surgical Colorectal Audit, no neoadjuvant therapy was used in 26% of cT2 patients aged > 75 years with rectal carcinoma in comparison with 14% in younger patients[18]. Furthermore, there is still a debate about adjuvant chemotherapy for rectal cancer. Future research about the balance between the oncological benefit, i.e., relative risk reduction of 50% in local recurrences and the side effects, i.e., relative risk increase of 50% in acute-treatment-relate toxicity, and long-term anorectal and sexual dysfunction[19-21] of neoadjuvant radiotherapy will help to determine the position of pretreatment dependent tissue-based markers like the TSR in predicting an individual prognosis.

It would be of interest to analyze the TSR and its prognostic value in biopsy specimens of well described areas of a rectal tumor. Prognostic information could then be more accurate by the use of tumor or stroma specific stainings. Besides, it would be desirable to develop a more objective instrument to determine the TSR than the visual estimation in this study. This could be provided by the use of tumor specific staining and the development of computer software in the growing field of digital pathology.

Determination of the TSR has the potential to identify patients without lymph node metastasis with a good and a poor clinical outcome and can thereby help in decision making on (neo)adjuvant treatment policy in individual cases. Determination of the TSR in routine sections is feasible and can be done with a good concordance by different observers.

COMMENTS

Background
Colorectal cancer is one of the most common form of cancer in both men and women. The TNM staging system, the most common system to stage colorectal tumors, is used to discriminate between patients with a better and a poor prognosis, but it seems insufficiently able to predict the prognosis of the individual patient. Additional prognostic factors are desirable, because a part of the patients is overtreated and consequently exposed to a higher risk on therapy related complications. The tumor-stroma ratio (TSR), the proportion of carcinoma relative to the proportion of tumor stroma in the histopathological section through the tumor, has proven to be of prognostic value in several malignancies.

Research frontiers
A previous study, on a small number of rectal cancer patients, showed that a high TSR is possibly related to both a longer disease free and overall survival. In this respect, it is meaningful to explore the relevance of the TSR in a larger cohort of patients with rectal adenocarcinoma, as the authors did.

Innovations and breakthroughs
This paper showed that the TSR has potential as a prognostic factor for survival in surgically treated rectal cancer patients, especially in lymph node negative cases. The effect of a high TSR on survival demonstrated in this study is in line with other studies on the prognostic impact of the TSR in colorectal carcinomas and other malignancies. The present study is however the first that has identified a subgroup of patients with rectal cancer, namely lymph node metastasis negative patients with an intermediate TSR, whereby the TSR is a strong prognostic factor.

Applications
The determination of the TSR may contribute to stratify patients for prognosis and has the potential to contribute to decision making regarding the individual treatment policy in rectal cancer.

Terminology
The TSR is the proportion of carcinoma relative to the proportion of tumor stroma in the histopathological section through the tumor. The carcinoma percentage (CP) is a derivative of the TSR and is complementary to the percentage of stroma and other components, like mucus. TSR-low including the CP-values ≤ 30%, TSR-intermediate including the CP-values 40%, 50% and 60%, and TSR-high including the CP-values ≥ 70%.

Peer-review
The study is well designed and clearly presented and the topic of high interest for oncologists that should decide after surgery what patients will benefit more from an adjuvant treatment.

REFERENCES
11 Koch GG, Landis JR, Freeman JL, Freeman DH Jr, Lehnen RC. A
general methodology for the analysis of experiments with repeated measurement of categorical data. *Biometrics* 1977; 33: 133-158 [PMID: 843570]


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