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The stromal part of adenocarcinomas of the oesophagus: Does it conceal targets for therapy?

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ABSTRACT

Objective: To evaluate the prognostic value of the tumour stroma ratio (TSR) in resected adenocarcinoma of the oesophagus.

Background: In the literature, a refinement of oesophageal cancer staging has been proposed. Recently, TSR has been identified as a histological characteristic of the tumour itself that proved to be a strong predictor for survival in colorectal cancer.

Methods: In our cancer registry database, we identified 93 consecutive patients who underwent resection for oesophageal adenocarcinoma between 1990 and 2004 in two hospitals in our region. Using a predefined histopathological protocol, TSR was determined on the original haematoxylin–eosin (H&E) tissue sections of oesophagectomy specimens by two independent investigators.

Results: With a cut-off value of 50% tumour/stroma, patients were classified as TSR high ($n = 60$) or TSR low ($n = 33$). There were no significant differences in patient, tumour and treatment characteristics between the two groups, except for M status (M1a) and radicality of resection. The (disease-free) survival in the TSR high group was significantly better than in the TSR low group. By multivariate analysis, TSR was identified as a highly significant prognostic factor for overall survival (HR 2.0; $P = 0.010$), independent of depth of tumour invasion, nodal status, lymph node ratio, extracapsular involvement, TNM stage, histological grade and radicality of resection.

Conclusion: TSR is a new and practicable prognostic tumour characteristic for oesophageal adenocarcinoma that can discriminate patients with a poor outcome from those with a better outcome.

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1. Introduction

Traditionally, the method for staging oesophageal cancer is according to the TNM-system of the International Union against Cancer (UICC) describing the extent of the primary tumour (T), the absence or presence of metastasis to regional lymph nodes (N) and the absence or presence of distant metastasis (M).[1] In the literature, there is an ongoing debate about revision of the current TNM staging model for oesophageal cancer.[1–3] Length of the tumour, depth of infiltration and the number and ratio of involved lymph nodes can give a better prognostication for patients after resection for oesophageal cancer.[4–7]

For different types of tumours, extensive research is performed to distinguish patients with low and high risk profiles on the basis of molecular techniques.[8] Methods aimed at genomic expression analysis using array technology or proteomics have not yet led to a clear set of prognostic factors that can be used for individual patient management.[9] New methods that select patients with a high risk for tumour recurrence after resection of the primary tumour could improve the efficacy of (neo)adjuvant therapies.

The tumour micro-environment plays a crucial role in the progression, growth and spread of cancers.[10–12] Only recently, the amount of stroma in direct relation to the tumour has been linked to survival by our group of investigators.[13,14] In a series of 122 colon cancers, the percentage of tumour cells compared to the stroma surrounding them, determined on standard haematoxylin and eosin (H&E) sections, proved to be an independent prognostic factor for overall survival and disease-free survival. To our knowledge, such relation has never been explored for adenocarcinoma of the oesophagus.

The purpose of this study was to evaluate the prognostic value of tumour stroma ratio (TSR) in oesophageal adenocarcinoma, and its relationship with other prognostic factors.

2. Methods

2.1. Patients and tissue samples

From the database of the Comprehensive Cancer Centre Leiden (CCCL), we selected all patients with oesophageal adenocarcinoma who underwent resection with curative intent between 1990 and 2004 at the Department of Surgery of the Leiden University Medical Centre (LUMC) or the Reinier de Graaf Gasthuis in Delft (RdGG). Patients who were treated with neoadjuvant therapy were excluded, as were patients who died within 30 d after surgery. Patient, tumour and treatment characteristics were retrieved from the original patient files and checked for inconsistencies with the data from the CCCL. Original pathological reports were reviewed; any discrepancies with the CCCL database were checked on the original patient material by a pathologist (VS). For all included patients, the H&E-stained sections from the primary tumour in the surgical specimen were retrieved from the Department of Pathology of the respective hospital. All samples were handled in a coded fashion, according to National ethical guidelines ('Code for Proper Secondary Use of Human Tissue', Dutch Federation of Medical Scientific Societies).

2.2. Histopathological protocol

On microscopic examination, the 5 μm H&E-stained sections from the primary tumour were routinely analysed, and the section showing the most invasive part was selected. From this section, the TSR was visually estimated on the basis of morphological characteristics. The assessment was done on the basis of the analysis of at least one microscopic field using a 10× microscope objective (100× total magnification). In case of tumour heterogeneity, those areas with the lowest TSR were considered to be of worse prognostic value and therefore deemed decisive. Stromal tissue not containing any tumour cells was considered not to have an apparent relation to the tumour. The estimate was then recorded as the TSR. A 50% cut-off value was used as earlier described by Mesker and colleagues[13], who determined the optimal threshold level of TSR to be 50%. Using this protocol, the sections from the LUMC-patients and RdGG-patients were independently assessed by two investigators (MT and VS or JSN). In those cases where the two researchers disagreed, the judgement of a third, unrelated pathologist (Dr. A.M. van Leeuwen) was decisive. TSR was defined as TSR low (i.e. <50%) or TSR high (i.e. $\geqslant$50%). TNM staging was done according to UICC guidelines.

2.3. Follow-up

Follow-up data were collected until death or July 2009. All patients had a regular follow-up schedule consisting of 3-monthly visits during the first 2 years after surgery and 6-monthly visits thereafter. Routine radiological examinations were not performed. When necessary, the patient's general practitioner was contacted for additional information.

2.4. Statistics

Statistical calculations were performed using SPSS version 15.0 (Statistical Package for the Social Sciences, Chicago, IL). Differences in patient, tumour and treatment characteristics as well as outcome measurements were assessed using the Mann–Whitney test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Survival was calculated from the date of surgery using the Kaplan–Meier method. For the analysis of overall survival, events were defined as death from any cause. For the analysis of disease-free survival, the events were defined as first loco-regional or distant tumour recurrence or death from any cause. Differences in survival distributions were tested with log rank statistics. The Cox proportional hazards model was used to determine the hazard ratio (HR) of explanatory variables on overall survival and disease-free survival.

Variables achieving a probability value of less than 0.05 in the univariate analysis were subsequently introduced in a multivariate stepwise proportional-hazard analysis (Cox model) to identify those variables significantly associated with tumour stroma ratio. The results are given as hazard ratios with their 95% confidence interval (CI). *P*-values < 0.05 (2-sided) were considered statistically significant.

3. Results

3.1. Patient demographics

Ninety-three patients were included in the study; a consecutive series of 55 patients from the LUMC and 38 patients from the RdGG. Overall, 74 men (80%) and 19 women (20%) were included, with a median age of 64 (range 37–85) years at the date of surgery.

3.2. Histopathology

On microscopic evaluation, the sections from the resected tumours showed a wide variety in TSR, ranging from very solid tumours with almost no stromal involvement to tumours with large areas of stromal proliferation interspersed with single and grouped tumour cells (Fig. 1). We evaluated a total of 275 sections from 93 resected tumours. For 29 patients, TSR was estimated on one section derived from the most invasive part of the tumour. For the other 64 patients, more sections were available to search for the tumour area with the lowest TSR, and the section showing the most invasive part was selected. A large difference in the number of areas with a low TSR (if present) was observed, ranging from only one microscopic field (100× total magnification) up to more than five. In general, the lowest ratios were found near the site of deepest microscopic infiltration. Assessed by two independent investigators, 33 tumours had a low TSR and 60 a high TSR. In seven cases (8%) there was no agreement between the investigators; in those cases, the observation of a third investigator was decisive (kappa value 0.835).

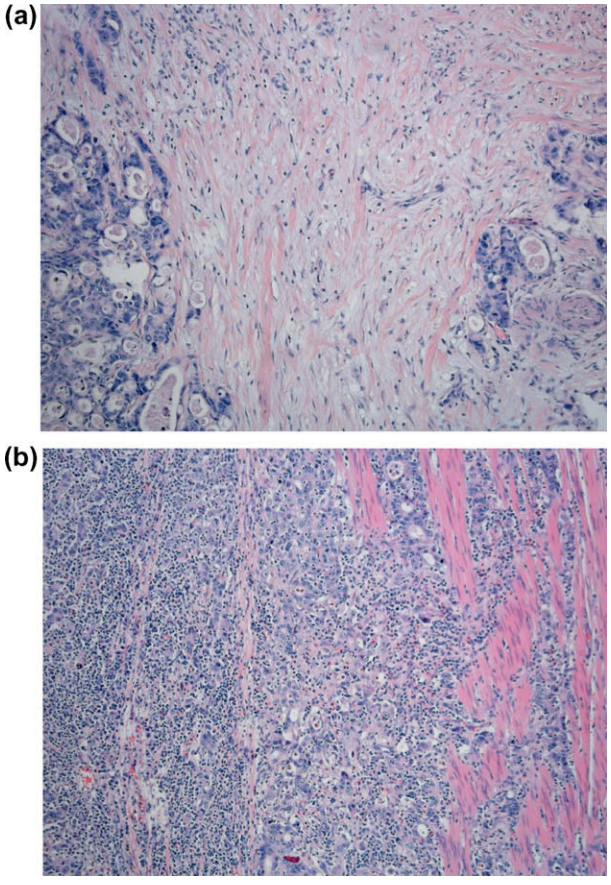


Fig. 1 – H&E-stained 5 μm sections of oesophageal adenocarcinoma. (a) Tumour with large area of stromal proliferation interspersed with single and grouped tumour cells (example of TSR low). (b) Solid tumour with almost no stromal involvement (example of TSR high). *Abbreviations:* H&E: haematoxylin and eosin; TSR: tumour stroma ratio.

3.3. Correlation of TSR with other prognostic parameters

Table 1 shows patient, tumour and treatment characteristics for the TSR low (less than 50% tumour cells) and the TSR high (50% tumour cells or more) groups. There were no significant differences between the two groups, except for M status and radicality of the resection. All three patients with M1a disease were in the TSR low group, and the tumours were less often resected with tumour-free margins in this group.

Follow-up was complete. Median follow-up for all patients was 23 months (range 3–220 months). The 3-year overall survival rates and disease-free survival rates were 19% and 12% in the TSR low group, and 53% and 43% in the TSR high group, respectively. Median overall survival for patients with a low TSR was 16 months (95% CI 13–19 months) compared to 42 months (95% CI 17–68 months) for patients with a high TSR. Survival analysis showed that this difference was highly significant ($P < 0.001$) with a hazard ratio of 2.0 for TSR low group (Fig. 2a). For disease-free survival, similar results were found (Fig. 2b).

In the univariate model, T status, N status, lymph node ratio, extracapsular lymph node involvement, TNM stage, differentiation grade and radicality of resection were also significantly related to both overall survival and disease-free survival (Table 2a). In multivariate analysis, TSR remained an independent prognostic variable for overall survival as were extracapsular lymph node involvement and radicality of resection (Table 2b).

Survival curves for stages I–IIa patients are shown in Fig. 3. The difference between the TSR high group and the TSR low group remained statistically significant in this subgroup of patients. In the univariate analysis of overall survival and disease-free survival, the hazard ratios for TSR were 2.8 (95% CI 1.3–6.2; $P = 0.008$) and 2.5 (95% CI 1.1–5.5; $P = 0.019$), respectively. For patients with stages III–IVa disease, TSR had no significant influence on overall survival ($P = 0.075$) or disease-free survival ($P = 0.076$).

4. Discussion

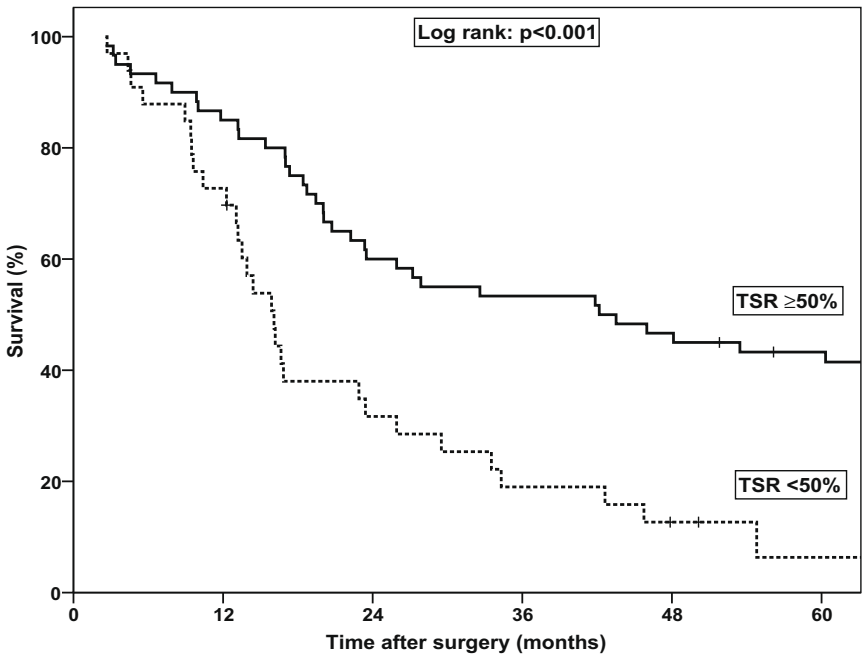
To our knowledge, the present study is the first report on the prognostic value of TSR in oesophageal adenocarcinoma. The results show that classification of oesophageal adenocarcinoma according to TSR provided highly significant prognostic information. The hazard ratios for overall survival and disease-free survival were of comparable magnitude as those of many traditional prognostic factors such as lymph node involvement and differentiation grade. Also in the group of node-negative patients (stages I and IIa), TSR differentiated between a poor and a better survival.

Table 1 – Patient, tumour and treatment characteristics for 93 patients who underwent oesophageal resection for adenocarcinoma, grouped by tumour stroma ratio (TSR).

Characteristics	Total (n = 93)		TSR < 50% (n = 33)		TSR ⩾ 50% (n = 60)		P-value
	No. of patients	%	No. of patients	%	No. of patients	%	
Gender							0.69
Male	74	80	27	82	47	78	
Female	19	20	6	18	13	22	
Median age at surgery in years (range)	64	37–85	67	37–82	62	42–85	0.086
Median tumour length in mm (range)	40	5–100	45	10–100	30	5–98	0.157
Tumour location							
Mid 1/3	1	1	0	0	1	2	
Distal 1/3	47	51	18	55	29	48	
GEJ	45	48	15	46	30	50	
Surgical approach							0.674
Transhiatal	70	75	24	73	46	77	
Transthoracic	23	25	9	27	14	23	
pT status							0.061
pT1	16	17	2	6	14	23	
pT2	17	18	5	15	12	20	
pT3	60	65	26	79	34	57	
pN status							0.266
pN0	41	43	12	36	29	48	
pN1	52	57	21	64	31	52	
Lymph node ratio							0.267
<0.2	55	59	17	52	38	63	
⩾0.2	38	41	16	49	22	37	
Extracapsular LNI							0.496
Node negative	41	44	12	36	29	48	
No extracapsular LNI	33	36	14	42	19	32	
Extracapsular LNI	19	20	7	21	12	20	
pM status							**0.042**
pM0	90	97	30	91	60	100	
pM1a	3	3	3	9	0	0	
pTNM stage							0.074[a]
I	12	13	2	6	10	17	
II	39	42	12	36	27	45	
III	39	42	16	49	23	38	
IV	3	3	3	9	0	0	
Differentiation grade							0.231
Well	8	9	5	15	3	5	
Moderate	48	52	15	46	33	55	
Poor	37	40	13	39	24	40	
Radicality							**0.010**[b]
R0	66	71	18	55	48	80	
R1	20	22	10	30	10	17	
R2	7	8	5	15	2	3	
Adjuvant therapy							0.286
No	90	97	31	94	59	98	
Yes	3	3	2	6	1	2	

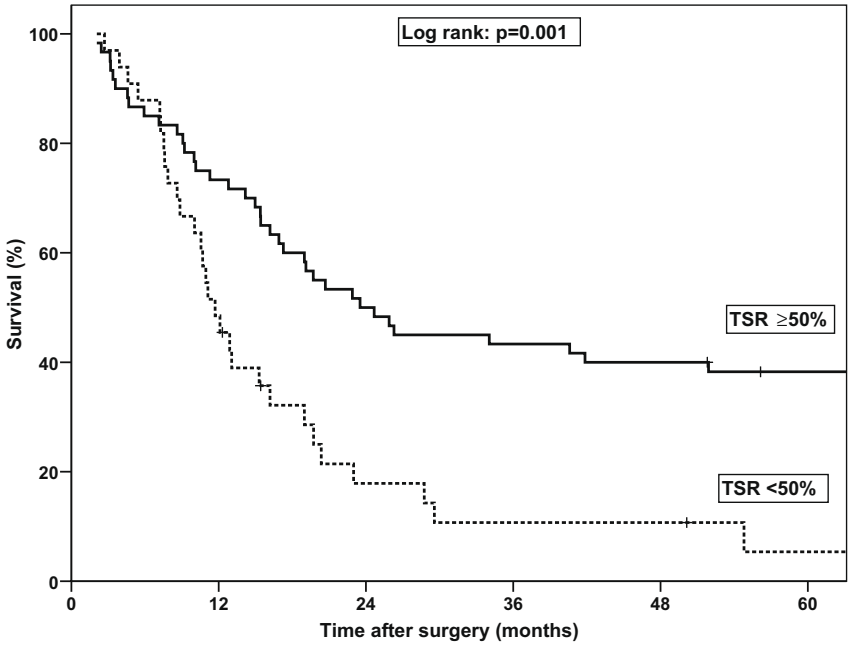
Abbreviations: GEJ: gastro-oesophageal junction; TNM: tumour node metastasis; LNI: lymph node involvement.
a Chi-square comparing stages I–II versus stages III–IV.
b Chi-square comparing R0 versus R1–R2.

The determination of TSR proved to be a quick and simple procedure which can be performed during routine pathological examination, using standard H&E sections without the need for additional staining. Inter-observer agreement was high (kappa value 0.835). In only seven of 93 cases (8%), there was no agreement between the two investigators, and a third pathologist had to make the decision.



TSR ≥ 50%	60	51	36	32	28	24
TSR <50%	33	24	10	6	3	1
Total	93	75	46	38	31	25

Fig. 2a – Overall survival for all 93 patients who underwent oesophageal resection for adenocarcinoma; TSR high versus TSR low. The numbers in the box refer to the number of patients at risk at 12-month intervals.



TSR ≥ 50%	60	44	30	26	24	21
TSR <50%	33	16	5	3	3	1
Total	93	60	35	29	27	22

Fig. 2b – Disease-free survival for all 93 patients who underwent oesophageal resection for adenocarcinoma; TSR high versus TSR low. The numbers in the box refer to the number of patients at risk at 12-month intervals.

Table 2a – Univariate cox regression analysis for overall and disease-free survival in 93 patients who underwent oesophageal resection for adenocarcinoma.

	Univariate analysis					
	Overall survival			Disease-free survival		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
TSR			**0.001**			**0.002**
⩾50%	1.000	Ref.	–	1.000	Ref.	–
<50%	2.382	1.460–3.885	0.001	2.186	1.340–3.564	0.002
Surgical approach			0.088			0.068
Transhiatal	1.000	Ref.	–	1.000	Ref.	–
Transthoracic	1.550	0.937–2.562	0.088	1.596	0.966–2.639	0.068
pT status			**0.003**			**0.011**
pT1	1.000	Ref.	–	1.000	Ref.	–
pT2	1.243	0.526–2.938	0.619	1.516	0.641–3.582	0.343
pT3	2.815	1.400–5.661	0.004	2.664	1.328–5.346	0.006
pN status			**0.004**			**0.004**
pN0	1.000	Ref.	–	1.000	Ref.	–
pN1	2.096	1.275–3.444	0.004	2.073	1.259–3.413	0.004
Lymph node ratio			**<0.001**			**<0.001**
<0.2	1.000	Ref.	–	1.000	Ref.	–
⩾0.2	2.516	1.546–4.094	0.000	2.509	1.536–4.097	0.000
Extracapsular LNI			**0.001**			**0.001**
Node negative	1.000	Ref.	–	1.000	Ref.	–
No extracapsular LNI	1.698	0.977–2.950	0.060	1.659	0.954–2.886	0.073
Extracapsular LNI	3.309	1.772–6.180	0.000	3.456	1.841–6.487	0.000
pM status			0.279			0.117
pM0	1.000	Ref.	–	1.000	Ref.	–
pM1a	1.904	0.593–6.118	0.279	2.581	0.789–8.443	0.117
pTNM Stage			**0.014**			**0.017**
I	1.000	Ref.	–	1.000	Ref.	–
II	1.742	0.761–3.988	0.189	1.898	0.829–4.345	0.129
III	3.227	1.395–7.466	0.006	3.085	1.335–7.128	0.008
IV	4.182	1.053–16.612	0.042	5.923	1.460–24.025	0.013
Differentiation grade			**0.035**			**0.031**
Well	1.000	Ref.	–	1.000	Ref.	–
Moderate	0.844	0.354–2.015	0.703	0.939	0.394–2.239	0.887
Poor	1.621	0.669–3.924	0.284	1.811	0.749–4.376	0.187
Radicality			**<0.001**			**<0.001**
R0	1.000	Ref.	–	1.000	Ref.	–
R1	3.629	2.063–6.383	0.000	3.324	1.884–5.864	0.000
R2	6.895	2.916–16.301	0.000	6.267	2.693–14.586	0.000
Adjuvant therapy			0.136			0.234
No	1.000	Ref.	–	1.000	Ref.	–
Yes	2.434	0.755–7.841	0.136	2.027	0.634–6.489	0.234

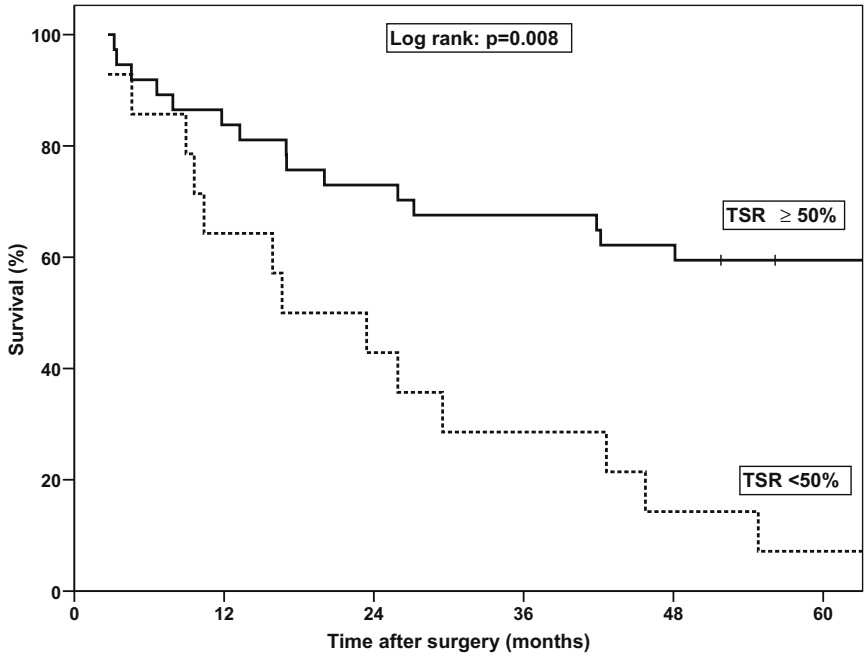
Abbreviations: HR: hazard ratio; CI: confidence interval; TSR: tumour stroma ratio; TNM: tumour node metastasis; LNI: lymph node involvement.

The use of TSR as a prognostic discriminator has recently been introduced in a paper by Mesker and colleagues.[13] In a study of 122 patients with stages I–III colon carcinoma, the concept of focusing on smaller portions of stromal proliferation within a tumour was developed. The optimal threshold level of TSR was determined on the basis of a maximum discriminating power for overall survival and disease-free survival. The 50% level was the most representative (more than TSR as a constant variable). Following this finding, it was decided in the present study to score TSR as <50% or ⩾50%. TSR in colon carcinoma was found to be an independent prognostic factor for survival. These results were confirmed in a study of 135 patients with stages I–II colon cancer.[14] Our hypothesis was that TSR might be an important prognostic factor in other types of adenocarcinomas. Two previous studies have evaluated the presence of increased stromal proliferation in oesophageal adenocarcinoma. In one study, 60% of 23 patients with oesophageal adenocarcinoma had an 'inflammatory stromal reaction', but no survival analysis was performed.[15] In the other study, a follow-up study of 96 patients, the presence or absence of a 'prominent desmoplastic tumour stroma' did not affect the survival significantly.[16]

Table 2b – Multivariate cox regression analysis for overall and disease-free survival in 93 patients who underwent oesophageal resection for adenocarcinoma.

	Multivariate analysis					
	Overall survival			Disease-free survival		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
TSR < 50%	2.006	1.181–3.407	**0.010**	1.553	0.923–2.611	0.097
T status (T3)	1.384	0.600–3.189	0.446	1.155	0.500–2.664	0.736
Lymph node ratio ⩾ 0.2	1.620	0.841–3.121	0.149	1.838	0.967–3.494	0.063
Presence of extracapsular LNI	2.469	1.324–4.606	**0.004**	2.897	1.562–5.372	**0.001**
TNM stage (III/IV)	0.826	0.369–1.851	0.643	0.822	0.362–1.864	0.638
Differentiation grade (Poor)	1.597	0.959–2.657	0.072	1.622	0.970–2.714	0.065
Irradical resection (R1/R2)	3.307	1.779–6.149	**<0.001**	3.755	1.988–7.090	**<0.001**

Abbreviations: HR: Hazard Ratio; CI: confidence interval; TSR: tumour stroma ratio; LNI: lymph node involvement; TNM: tumour node metastasis.

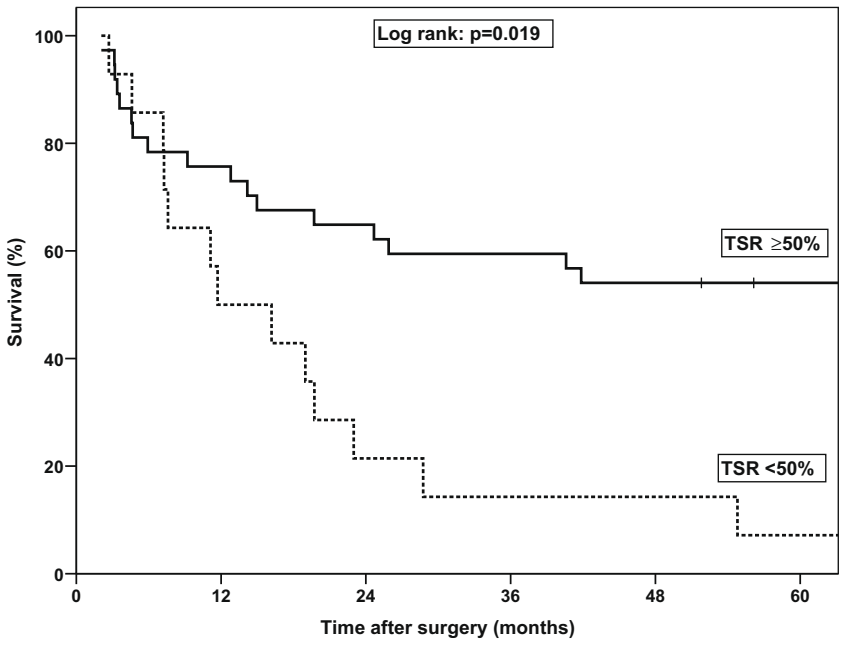


TSR ≥ 50%	28	23	21	19	18	15
TSR <50%	12	8	5	3	2	1
Total	40	31	26	22	20	16

Fig. 3a – Overall survival for patients with pathological stages I–IIA; TSR high versus TSR low. The numbers in the box refer to the number of patients at risk at 12-month intervals.

In a review article by Lagarde and colleagues, several histopathological factors with a strong prognostic value for oesophageal adenocarcinoma were identified.[17] All these factors were included in our study and had significant prognostic impact in univariate analysis. However, TSR showed a hazard ratio that was larger than most of these well-known prognostic factors. In multivariate analysis, TSR proved to be an independent factor.

TSR and its relation to prognosis may be explained pathophysiologically. In 2007, Tsujino and colleagues have suggested that the abundance of myofibroblasts in cancer-associated stroma is an indicator for poor survival after colorectal surgery.[18] This supports our observation that stromal components not only are supportive, but also could play a role in the maintenance and growth of tumours. They receive and elicit oncogenic signals and undergo quantitative and morphological changes.[19] One of the key mediators in the crosstalk between the tumour and its micro-environment is transforming growth factor β (TGF-β), the cytokine believed to be responsible for the proliferative change in fibroblasts.[12] An increased production of TGF-β1 stimulates the synthesis of extracellular matrix proteins and chemo attraction of



TSR ≥ 50%	28	21	19	17	16	14
TSR <50%	12	6	3	2	2	1
Total	40	27	22	19	18	15

Fig. 3b – Disease-free survival for patients with pathological stages I–IIA; TSR high versus TSR low. The numbers in the box refer to the number of patients at risk at 12-month intervals.

fibroblasts and it has a negative impact on survival.[20–22] Koliopanos and colleagues found that increased mRNA-expression of CTGF (connective tissue growth factor), a downstream mediator of TGF-β1, influenced survival negatively in oesophageal adenocarcinoma, although it was not associated with an increase in stroma cells.[23] It is thought that growth factors such as TGF-β1 and CTGF may mediate the interactions between tumour and stroma.[18] Our study underlines this hypothesis that an increased amount of stromal involvement, even when it is detected in only a small part of the total tumour mass, can be linked to an unfavourable prognosis independent of other prognostic parameters. Possibly, this particular section of the tumour finds itself in a tumour-host micro-environment in which the host participates in the expansion of neoplastic cells. Tumour cells can alter their adjacent stroma by producing stroma-modulating growth factors to form a supportive environment for tumour progression. Therefore, in patients with a low TSR, the presence of more stroma in the tumour may have resulted in the production of factors that promote tumour progression and, consequently, in a higher rate of R1 resections.[24]

If better understood, the pathological communication between tumour cells and host cells could be used as a target for new cancer therapies, especially in node-negative patients (stages I and IIa). In these patients, but not in patients with locally advanced tumours, TSR was an independent predictor of survival after surgery. Few studies have focused on node-negative oesophageal cancer patients. As a consequence there has been no comprehensive evaluation of the influence of histopathological characteristics on survival in this subgroup.[25] Our results illustrate that patients with N0 disease had a diverse outcome when differentiated for TSR. Following these findings, TSR could have additional value for targeting (new) adjuvant therapies in resected oesophageal adenocarcinoma. An analysis of H&E sections from studies on adjuvant therapies for oesophageal adenocarcinoma may provide further evidence for this theory.

Although our study population was homogenous (only patients who underwent oesophageal resection for adenocarcinoma without neoadjuvant therapy), the study has its shortcomings. First, this was a relatively small retrospective study of 93 patients spanning a large time period. Second, the histopathological analysis was done on H&E-stained sections of oesophagectomy specimens. It would be of interest to analyse biopsy specimens in the future. Since neoadjuvant strategies are increasingly being developed, information could then be provided before the start of neoadjuvant treatment. A prospective biopsy study is now ongoing at our institutes. In this study, TSR will be scored as a continuous variable (rather than TSR low and TSR high) to find the best discriminating value in biopsy specimens.

In summary, we have shown that a low TSR in resected oesophageal adenocarcinoma was associated with a poor long-term survival. TSR can be determined easily during routine pathological examination on H&E-stained sections of the resected tumour. Implementing this novel parameter could

have additional value in identifying patients at increased risk for early tumour recurrence or metastatic disease, i.e. those patients who do not benefit from surgical resection alone. Furthermore, our study results emphasise that the stromal part of a tumour is related to outcome and should therefore also be explored in the search for new targeted therapies.

Conflict of interest statement

None declared.

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