The tumor-stroma ratio in epithelial cancer types: towards implementation in diagnostic pathology

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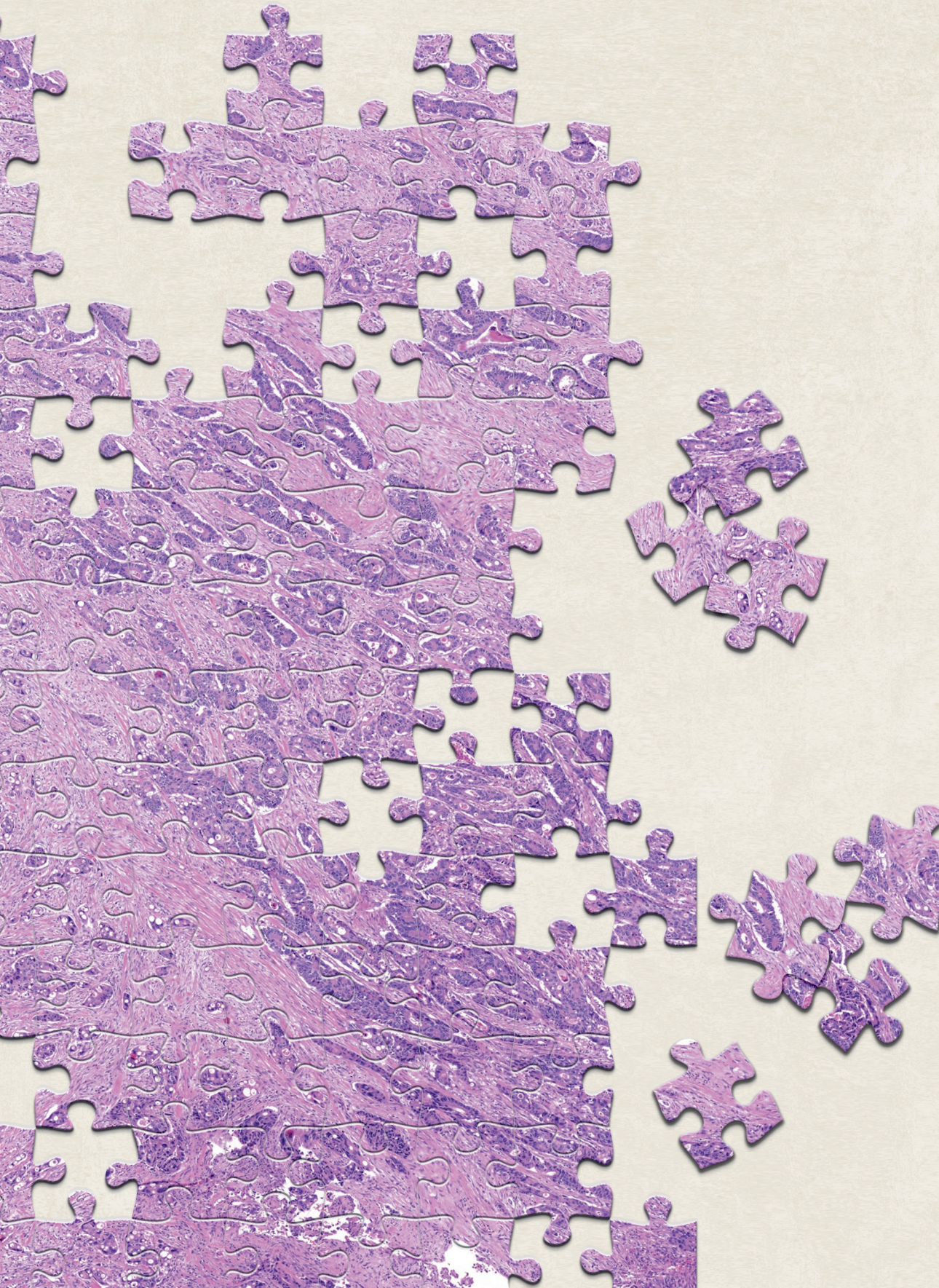

CHAPTER 9

The prognostic value of the tumor-stroma ratio in squamous cell lung cancer, a cohort study

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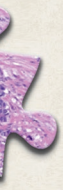

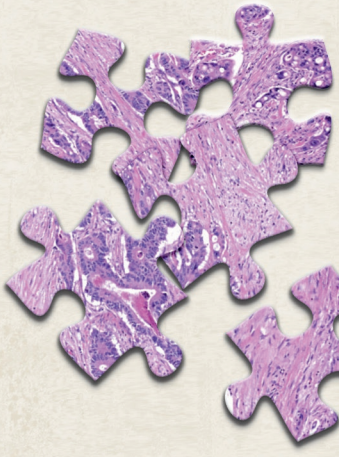

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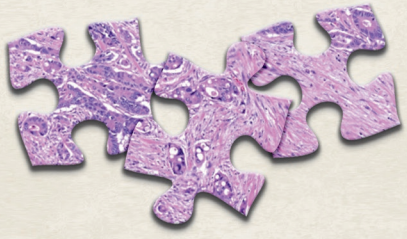

Abstract

Objectives: The tumor-stroma ratio (TSR) is based on the relative amount of stroma in the primary tumor and has proven to be an independent prognostic factor in various solid tumors. The prognosis of patients and adjuvant treatment decision making in lung squamous cell carcinomas (SqCC) is based on the TNM classification. Currently, no other prognostic biomarkers are available. In this study we evaluated the prognostic value of the TSR in lung SqCC.

Material and Methods: Patients undergoing lung surgery because of lung SqCC between 2000 and 2018 at the Leiden University Medical Center were included. The TSR was scored on hematoxylin & eosin stained tissue sections. Based on the amount of tumor-stroma, two groups were defined: ≤50% was classified as a stroma-low tumor and >50% as stroma-high. The prognostic value of the TSR was determined with survival analysis.

Results: A total of 174 stage I-III patients were included. Of them, 79 (45%) were stroma-low and 95 (55%) stroma-high. Separately analyzed for tumor stages, the TSR showed to be an independent prognostic biomarker in stage II (n=68) for 5-year overall survival (HR=3.0; 95% CI, 1.1-8.6; p = 0.035) and 5-year disease free survival (DFS) (HR=3.6; 95% CI, 1.3-9.9; p = 0.014). Patients with a stroma-high tumor had a worse 5-year DFS in the whole cohort (HR 1.6; 95% CI, 1.0-2.4; p = 0.048), but no independent prognostic value was found.

Conclusion: In stage II lung SqCC patients, stroma-low tumors have a better prognosis compared to stroma-high tumors. Moreover, adjuvant chemotherapy could be spared for these stroma-low patients.

Introduction

Despite successful implementation of immunotherapeutic regimes in non-small cell lung cancer (NSCLC) treatment, NSCLC still has a poor prognosis with a 5-year overall survival rate of 17% (1). Although all types of lung cancer are associated with smoking, in squamous cell carcinoma (SqCC) the association is stronger than in adenocarcinomas, with 90-95% of the cases being associated (2, 3). SqCCs are thought to arise from metaplastic bronchial epithelium, and generally develop more solid and more centrally located in the lung in contrast to adenocarcinoma's (4). Moreover, SqCC has limited targetable options, as EGFR (epidermal growth factor receptor), BRAF or HER2 (human epidermal growth factor receptor 2) are rarely mutated in this tumor type (5).

The last few decades, research is focusing on the discovery of prognostic factors that complement the TNM-classification, to enable better risk-assessment with respect to survival and disease recurrence after initial surgery or radiotherapy. Many of such have been identified, varying from clinical and pathological factors (such as mitotic index (6, 7), micropapillary growth patterns in adenocarcinoma (8)), to molecular factors (such as EGFR, ALK (anaplastic lymphoma kinase) or K-RAS (Kirsten Rat Sarcoma) (5)). In SqCC lung tumors only few biological parameters have been identified, and none of them have been implemented into daily clinical practice.

Prior studies of other tumor types, e.g. breast cancer, colon cancer, and esophageal cancer (9), have demonstrated that the relative amount of tumor-stroma (the tumor-stroma ratio (TSR)) in the primary tumor is a robust biomarker for overall survival (OS) and disease free survival (DFS). The TSR is scored on hematoxylin and eosin (H&E) stained sections (10). Patients with a stroma-high tumor (>50% stroma) are proven to have a worse outcome compared to patients with a stroma-low (≤50% stroma) tumor (11, 12). In squamous cell lung cancer however, the TSR has not yet been validated as an independent prognostic factor. Although there are a few studies showing that stroma-high tumors have a worse prognosis in NSCLC (13-15), another study indicated that stroma-low tumors have a worse prognosis in patients with adenocarcinomas (16).

In the current study the independent prognostic value of TSR in lung SqCC is studied. Since there are no prognostic factors other than the TNM classification available in daily pathology practice for lung SqCC, there is a clear clinical need to identify these. A strong candidate is the TSR, as this is easily determined during routine diagnostics (in 1-2 min), reproducible and low in costs (10). Our hypothesis is that patients with a stroma-high lung SqCC have a worse OS and DFS compared to patients with a stroma-low tumor.

Materials and methods

Patient population

In this retrospective cohort study, patients diagnosed with SqCC of the lung undergoing lung surgery between January 2000 and January 2018 at the Leiden University Medical Center (LUMC) were enrolled. The H&E stained slides and the standard tumor characteristics were collected from the Department of Pathology. Patients were excluded when one of the following criteria was met: (I) an oncological history 10 years prior to surgery (except for basal cell carcinoma or cervical carcinoma in situ), (II) a diagnosis of stage IV lung cancer, (III) received neoadjuvant treatment, (IV) no R0 resection, and (V) death or recurrence within 3 months after surgery. All inclusion and exclusion criteria are shown in ***Supplementary Table 1***.

Histopathology

Routine diagnostic 4 µm H&E stained slides of the primary tumor resected during lung surgery were selected for analysis using a conventional light microscope. With a 2.5x or 5x objective (25x or 50x magnification), the area appearing to have the highest amount of stroma was selected. In this area of interest, a field was chosen with the highest stroma percentage and inspected with the use of a 10x objective (100x magnification). Tumor cells had to be present on all four sides of the vision field. The stroma percentage was scored per tenfold (for example, 10%, 20%, etc.) (10). For the statistical analysis the stroma percentages were categorized with the cut-off of 50%: stroma-low was defined as ≤50% and stroma-high as >50% (17). The TSR was independently estimated by two observers (MS and MP), and if the two observers disagreed, a third investigator (DC) was consulted. Representative images from a stroma-low and a stroma-high tumor are shown in ***Figure 1*.**

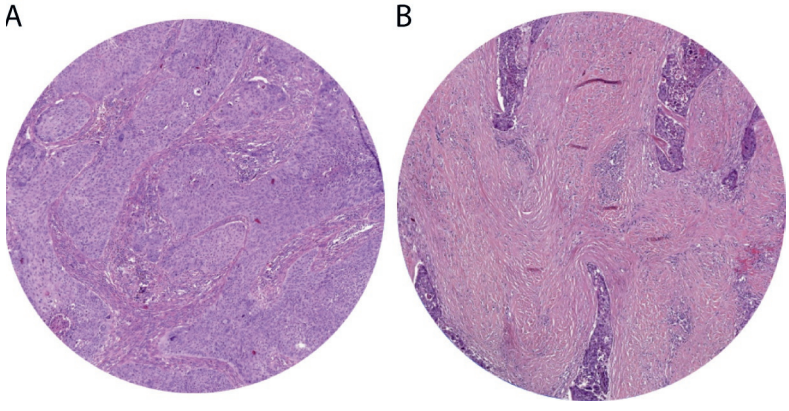

Figure 1. Examples of the 4µm hematoxylin & eosin stained slides of lung squamous cell carcinomas (100x magnification). A) A stroma-low squamous cell tumor. B) A stroma-high squamous cell tumor.

Statistical analysis

Student's T-test and Chi-squared test were used to compare the patient and tumor characteristics at baseline. The interobserver agreement for scoring TSR was analyzed with the use of Cohen's kappa coefficient. OS was defined as the time between the date of surgery and the date of death or the end of follow-up. DFS was defined as the time between the date of surgery and the date of death, the appearance of tumor progression, recurrence or the end of follow-up. The OS and DFS curves were drawn by Kaplan-Meier survival analysis, log-rank tests were used to compare the survival curves. In this study the 5-year OS and 5-year DFS were used for the analysis. The hazard ratio (HR) and corresponding 95% confidence interval (95% CI) for OS and DFS were assessed by univariate and multivariate Cox regression models. The survival analysis was performed for all pathological TNM (pTNM) stages. Statistical tests were 2-sided and p-values <0.05 were considered to be significant. SPSS version 25.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis.

Results

Patient characteristics

A total of 328 patients with lung SqCC underwent surgery at the LUMC between January 2000 and January 2018, and 174 patients met the inclusion criteria ***(Supplementary Figure 1)***. Of these 174 patients, 141 (81%) were male. The mean age at the date of surgery was 66 years, ranging from 45 – 83 years. The median tumor size was 4.75 cm with a range of 0.50 – 15.00 cm. Based on the pathological data, 47 (27%) patients had a stage I tumor, 68 (39%) patients a stage II tumor and 59 (34%) patients a stage III tumor. All baseline characteristics are shown in ***Table 1***.

Based on the amount of stroma, 79 (45%) patients were classified as stroma-low and 95 (55%) patients as stroma-high. There were no significant differences between the two groups at baseline ***(Table 1)***.

Table 1. Patients and tumor characteristics of 174 patients with lung squamous cell cancer.

Characteristics	Total n=174 (100%)	Stroma-low n=79 (45%)	Stroma-high n=95 (55%)	p-value
Age (years), mean (SD)	65.96 (8.54)	66.13 (9.07)	65.82 (8.12)	0.815
range	45-83			
Gender				
Male	141 (81)	61 (77)	80 (84)	0.241
Female	33 (19)	18 (23)	15 (16)	
Tumor size (cm),	4.75	4.40	5.00	0.076
median (range)	(0.50-15.00)	(0.50-15.00)	(1.40-13.00)	
pTNM stage				0.304
I	47 (27)	23 (29)	24 (25)	
II	68 (39)	34 (43)	34 (36)	
III	59 (34)	22 (28)	37 (39)	
pT status				0.457
T1	46 (26)	25 (32)	21 (22)	
T2	52 (30)	24 (30)	28 (30)	
T3	49 (28)	19 (24)	30 (32)	
T4	27 (16)	11 (14)	16 (17)	
pN status				0.860
N0	102 (59)	48 (61)	54 (57)	
N1	48 (28)	21 (27)	27 (28)	
N2	24 (14)	10 (13)	14 (15)	
Adjuvant therapy				0.495
No	121 (70)	57 (72)	64 (67)	
Yes	53 (31)	22 (28)	31 (33)	

Interobserver agreement.

In total, 22 (13%) slides needed a third review to reach complete agreement. The interobserver agreement with Cohen's kappa between the two observers for scoring the TSR showed a good agreement with a kappa of 0.747.

Survival analysis of the prognostic factors for the overall cohort

Although the Kaplan-Meier curve did not illustrate the hypothesized prognostic value of the TSR for the 5-year OS (log-rank OS, p = 0.179), it did for the 5-year DFS (log-rank DFS, p = 0.044) ***(Figure 2)***. The Cox univariate model showed that tumor size, pTNM stage and adjuvant therapy were significantly associated with the 5-year OS and DFS. Stroma-high patients had a significantly worse 5-year DFS compared to the stroma-low patients (HR=1.561; 95% CI, 1.004-2.426; p = 0.048), with a 5-year DFS of 42% versus 57%, respectively ***(Supplementary Table 2).*** Corrected in multivariate analysis for age, pTNM and adjuvant therapy, the TSR however showed no significance for 5-year DFS (HR = 1.366; 95%, 0.875-2.131; p = 0.170) ***(Table 2)***.

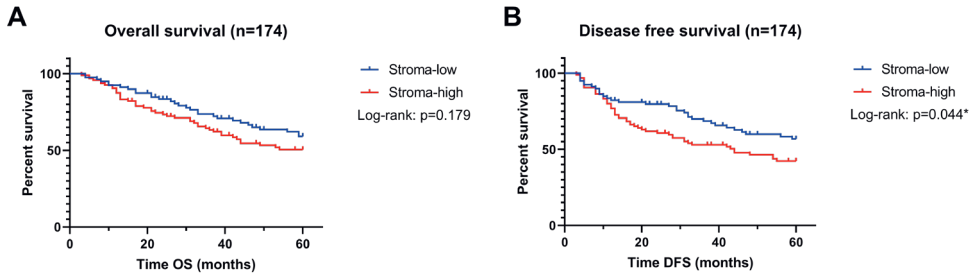

Figure 2. The 5-year survival curves of the 174 patients with squamous cell lung cancer. In A) the overall survival, log-rank p=0.190. In B) the disease free survival, log-rank p=0.046.

Table 2. Cox multivariate analysis for 5-year survival.

		Overall survival			Disease free survival		
	N (%)	HR	95% CI	p-value	HR	95% CI	p-value
TSR							
Stroma-low	79 (45)	REF		0.432	REF		0.170
Stroma-high	95 (55)	1.207	0.755-1.929		1.366	0.875-2.131	
Age	174	1.012	0.983-1.042	0.409	1.011	0.984-1.039	0.441
pTNM stage							
I	47 (27)	REF		<0.001	REF		<0.001*
II	68 (39)	0.614	0.316-1.191	0.149	0.562	0.302-1.049	0.070
III	59 (34)	2.005	1.083-3.713	0.027*	1.930	1.088-3.422	0.024*
Adjuvant therapy							
No	121 (70)	REF		0.210	REF		0.098
Yes	53 (31)	1.405	0.826-2.390		1.526	0.926-2.516	

HR: hazard ratio; CI: confidence interval; TSR: tumor-stroma ratio; REF: reference; pTNM: pathological tumor, node, metastasis stage; *: significant result.

Survival analysis per tumor stage

The Kaplan-Meier curves for stage II lung SqCC portray TSR as a significant prognostic factor for 5-year OS (log-rank OS, p = 0.025) and 5-year DFS (log-rank DFS, p = 0.007) ***(Figure 3)***. In stage II, patients with a stroma-high tumor showed a significantly worse 5-year survival compared to patients with a stroma-low tumor. The 5-year OS was 60% versus 84%, (stroma-high versus stroma-low) (HR= 3.045; 95% CI, 1.084-8.550; p = 0.035) and for 5-year DFS 54% versus 84% (HR=3.684; 95% CI, 1.337-10.156; p = 0.012), respectively. The univariate analysis did not differ for the clinicopathological characteristics when separately analyzed for stage. Corrected for age and adjuvant therapy in the multivariate analysis, the TSR remained a significant prognostic factor for OS (HR=3.001; 95% CI, 1.066-8.449; p = 0.037) and DFS (HR=3.594; 95% CI, 1.300-9.934; p = 0.014) ***(Table 3)***.

For stage I and III, the Kaplan-Meier curves showed no prognostic value ***(Supplementary figure 2 and 3)***, therefore the univariate and multivariate Cox regression models were not performed.

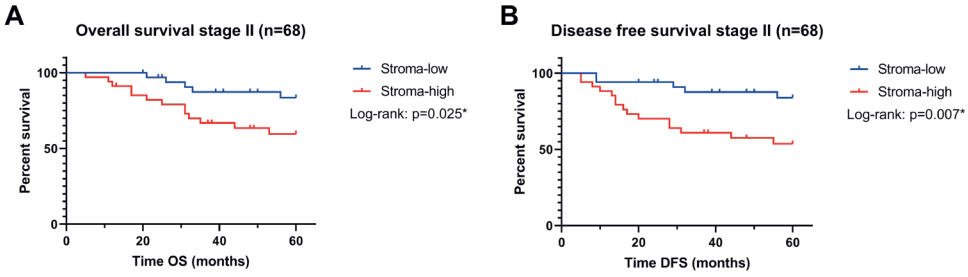

Figure 3. The 5-year survival curves of the 68 stage II patients with squamous cell lung cancer. In A) the overall survival, log-rank p = 0.022. In B) the disease free survival, log-rank p=0.005.

Table 3. Cox multivariate analysis for 5-year survival for stage II.

		Overall survival			Disease free survival		
	N (%)	HR	95% CI	p-value	HR	95% CI	p-value
TSR							
Stroma-low	34 (50)	REF		0.037*	REF		0.014*
Stroma-high	34 (50)	3.001	1.066-8.449		3.594	1.300-9.934	
Age	68	1.020	0.962-1.081	0.513	1.027	0.971-1.086	0.358
Adjuvant therapy							
No	47(69)	REF		0.133	REF		0.089
Yes	21 (31)	2.052	0.804-5.234		2.158	0.889-5.238	

HR: hazard ratio; CI: confidence interval; TSR: tumor-stroma ratio; REF: reference; *: significant result.

Discussion

This study aimed to evaluate the prognostic value of TSR in squamous cell lung cancer patients. Although the TSR could not be validated for the whole cohort, it did prove to be an independent prognostic factor in stage II lung SqCC for OS and DFS. Patients with a stage II stroma-high tumor had a significantly worse 5-year OS and DFS, compared to patients with a stage II stroma-low tumor. It is likely that patients diagnosed with a stage II tumor may receive adjuvant chemotherapy (18). Stroma-low stage II patients showed comparable survival rates to stage I patients, the latter not receiving adjuvant chemotherapy. Based on current results, it thus might be possible to spare patients with a stage II stroma-low tumor the adjuvant chemotherapy and any associated toxic side effects.

The TSR has proven to be an independent prognostic factor in various tumor types (9); solid tumors, e.g. breast cancer (12) and currently being validated in colon cancer (19), but also in

other squamous cell tumors as oral, laryngeal and (naso)pharyngeal cancer (20-22) and in all NSCLC patients (13, 15). However, it has not yet been validated in a single lung SqCC patient cohort with the microscopic method. Koike et al. (23) validated the prognostic value of the TSR scored by artificial intelligence in a SqCC lung cancer cohort. Our results are in accordance with previous research, and are of added value due to the homogenous group of SqCC lung tumors in which the TSR had not been previously investigated by microscopic assessment.

In the current study, our Kaplan-Meier survival curves showed that in the first 10 months after surgery the survival between the stroma-high and stroma-low patient groups was almost equal. This suggests that other factors are of influence for event occurrence other than tumor progression in the first months after surgery, as for example post-operative complications (24).

Since the introduction of immunotherapy and other targeted therapies in recent years, it gained an increasingly important role in the treatment of lung cancer patients. Mainly in patients with a low number of gene mutations, such as EGFR and ALK, targeted therapy shows positive effects. However, these are fairly new treatment options and this cohort is mostly from before the introduction of targeted therapy and immunotherapy, thus only a very small number of patients had these treatment opportunities. Future research may have to look into the association and/or predictive value of the TSR for the response to targeted therapy and immunotherapy.

Trends for treatment strategies for lung cancer are tending to move towards neoadjuvant treatment. Seeing the side effects of neoadjuvant therapy, prediction of the chance of responding is needed. In esophageal cancer, the TSR has also shown to be a predictive biomarker for the response to neoadjuvant treatment (25, 26). Therefore, it would also be interesting to investigate whether the TSR is predictive in lung cancer and whether it can be scored on biopsies. We anticipate this would be hard to study in lung biopsies however, because of the low and small amount of tissue received through lung biopsies sometimes only gained via cytology.

In conclusion, the TSR has an independent prognostic value in stage II lung SqCC for 5-year OS as well as for 5-year DFS, and this may lead to personalized treatment strategies when the TSR is determined during routine pathology. Suggesting that patients with a stage II stroma-low tumor may in future be spared from adjuvant therapy. Due to the relatively small sample size in this study, there is a need to validate this prognostic value in a larger cohort, preferably a prospective study.

Acknowledgments

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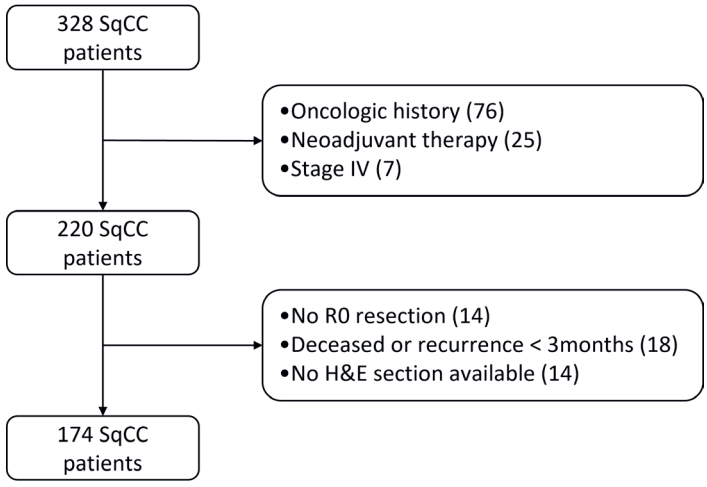
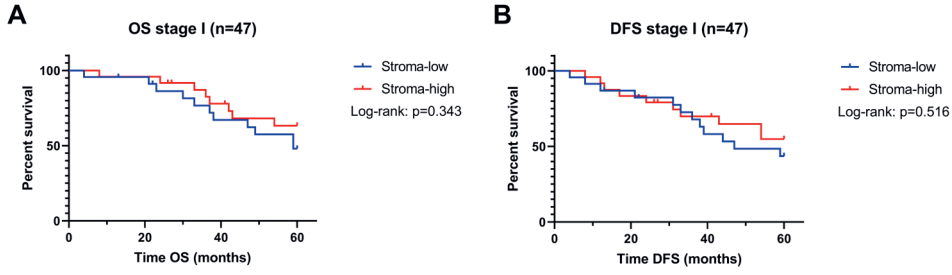
Supplementary material

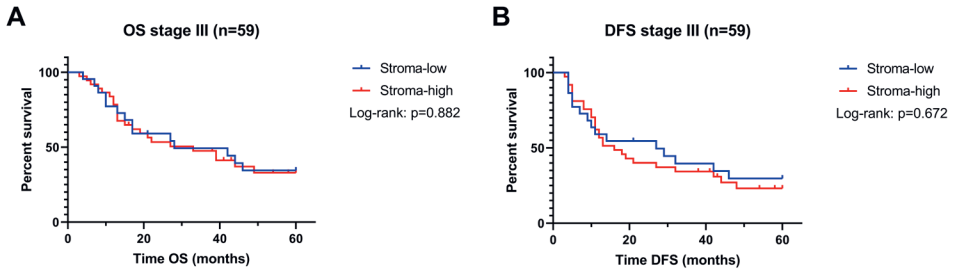


Supplementary Figure 1. Flowchart patient selection.

SqCC: squamous cell carcinomas; H&E: hematoxylin & eosin.



Supplementary Figure 2. A) Kaplan-Meier curve for the 5-year overall survival of 47 stage I patients with squamous cell lung cancer. Log-rank 5-year OS: p = 0.343. B) Kaplan-Meier curve for the 5-year disease free survival of 47 stage I patients with squamous cell lung cancer. Log-rank 5-year DFS: p=0.516.



Supplementary Figure 3. A) Kaplan-Meier curve for the 5-year overall survival of 59 stage III patients with squamous cell lung cancer. Log-rank 5-year OS: p = 0.882. B) Kaplan-Meier curve for the 5-year disease free survival of 59 stage III patients with squamous cell lung cancer. Log-rank 5-year DFS: p=0.672.

Supplementary Table 1. In- and exclusion criteria.

Inclusion criteria	Exclusion criteria
Age >18 years	Oncological history 10 year before diagnosis
Lung surgery between 2000 and 2018	(except for basal cell carcinoma and cervical
Squamous cell lung cancer	carcinoma in situ)
H&E slides available	Stage IV lung cancer
	Neoadjuvant therapy
	R1 or R2 resection
	Deceased or recurrence within 3 months after
	surgery

H&E: hematoxylin & eosin

Supplementary Table 2. Cox univariate analysis for 5-year survival.

		Overall survival			Disease free survival		
	N (%)	HR	95% CI	p-value	HR	95% CI	p-value
Age	174	1.009	0.981-1.037	0.544	1.006	0.980-1.032	0.668
Gender							
Male	141 (81)	REF		0.668	REF		0.445
Female	33 (19)	1.132	0.642-1.997		1.226	0.727-2.066	
Tumor size	174	1.090	1.000-1.188	0.049*	1.097	1.013-1.188	0.023*
pTNM stage							
I	47 (27)	REF		<0.001*	REF		<0.001*
II	68 (39)	0.648	0.340-1.235	0.188	0.603	0.329-1.106	0.102
III	59 (34)	2.347	1.346-4.093	0.003	2.360	1.405-3.964	<0.001*
pT status							
T1	46 (26)	REF		0.009*	REF		0.006*
T2	52 (30)	0.612	0.319-1.173	0.139	0.766	0.418-1.404	0.389
T3	49 (28)	1.039	0.563-1.918	0.902	1.180	0.657-2.118	0.580
T4	27 (16)	1.993	1.050-3.781	0.035*	2.286	1.235-4.232	0.008*
pN status							
N0	102 (59)	REF		<0.001*	REF		<0.001*
N1	48 (28)	0.828	0.463-1.478	0.523	0.842	0.487-1.454	0.537
N2	24 (14)	2.777	1.584-4.869	<0.001*	3.112	1.839-5.265	<0.001*
Adjuvant therapy							
No	121 (70)	REF		0.019*	REF		0.006*
Yes	53 (31)	1.760	1.099-2.818		1.859	1.195-2.892	
TSR							
Stroma-low	79 (45)	REF		0.183	REF		0.048*
Stroma-high	95 (55)	1.371	0.861-2.182		1.561	1.004-2.426	

HR: hazard ratio; CI: confidence interval; REF: reference; pTNM: pathological tumor, node, metastasis stage; pT: pathological tumor stage; pN: pathological node stage; TSR: tumor-stroma ratio; *: significant result.