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# Current clinicopathological challenges of lung cancer

**PhD Thesis**

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# INTRODUCTION

Lung cancer is an aggressive and frequent neoplastic disease worldwide, with an estimated 2.2 million new cases and 1.8 million deaths in 2020. Lung cancer is the leading cause of cancer mortality in men and behind breast and colorectal cancer, it is the third most frequent cause of cancer-related death in women. Despite complete surgical removal, the prognosis of lung cancer is generally poor [24], with recurrence rates of 15-30%, and 5-year OS rates of 60-70% [25]. However, lung cancer is not a homogeneous disease. There are several histological types with different prognosis and different treatment modalities. In our studies, we investigated three histological subtypes that are challenging in daily routine work: lung adenocarcinoma (1), the most common non-small cell lung cancer (NSCLC), pulmonary squamous cell carcinoma (2), the most smoking-related lung cancer, and pulmonary sarcomatoid carcinoma (3), a rare but therapeutically challenging lung neoplasm. In cases of pulmonary adenocarcinoma and squamous cell carcinoma, we evaluated different prognostic markers; while in sarcomatoid carcinoma, we analysed the impact of adjuvant chemotherapy on overall survival (OS).

## 1.1. THE MORE EXTENSIVE THE SPREAD THROUGH AIR SPACES, THE WORSE THE PROGNOSIS IS

Surgical resection is the standard therapy for lung adenocarcinoma, particularly in stage I. Due to the high incidence rate of adenocarcinoma, the prognostic factors are currently the subject of extensive research. Spread through air spaces (STAS) as a form of invasive tumour spread was described by Kadota and associates [18]. According to the classification of Thoracic tumours, published by the World Health Organization (WHO) in 2021, STAS is defined as “tumour cells within airspaces in the lung parenchyma beyond the edge of the main tumour” [21]. Presence of STAS is a significant risk factor for recurrence of small stage I lung adenocarcinoma in patients who underwent limited resection [18]; moreover, STAS significantly reduced the recurrence-free survival (RFS), OS and disease-free survival (DFS) in patients with resected adenocarcinomas of any stage in the categories of both extensive and limited STAS [19]. The presence of STAS has been extensively investigated but few publications have focused on the prognostic role of the extent of STAS. To date, only two research groups, Uruga and colleagues [22] and Morimoto and colleagues [23], have investigated the significance of the degree of STAS. The former research classified the extent of STAS semi-quantitatively and correlated the results with prognosis [22]. The former characterised the extent of STAS by the number of intra-alveolar tumour cell clusters, the latter by the presence of a so-called "free tumour cluster"

(FTC) [23]. The presence of FTC (>3 tumour cell clusters of less than 20 cells >3 mm from the main tumour body) is by definition indicative of more pronounced STAS. To our knowledge, the impact of the extent of STAS on survival and the reproducibility of the methodology have not been investigated outside these two publications.

## 1.2 THE PROGNOSTIC IMPORTANCE OF TUMOUR BUDDING, SINGLE CELL INVASION, AND NUCLEAR DIAMETER IN LUNG SQUAMOUS CELL CARCINOMAS

Prognostic markers for lung adenocarcinomas have been investigated in a wide range of studies recently. Such a prominent marker is the grading classification recommended by the International Association for the Study of Lung Cancer (IASLC) [77], which has since been included in the latest edition of the Thoracic tumours classification published by the WHO [26]. Far fewer publications focus on prognostic factors for pulmonary squamous cell carcinomas.

In pulmonary squamous cell carcinomas, tumour budding, minimal cell nest size, and nuclear diameter are considered as possible candidates for prognostic purposes [30-32]. Tumour budding is defined as the presence of isolated small tumour nests composed of less than 5 tumour cells at the invasive tumour front [33]. Firstly, tumour budding was introduced in colorectal cancer as a morphological feature, and its prognostic role has been validated [34-38]. Internationally accepted reporting and clinical implications were recommended at the International Tumour Budding Consensus Conference in 2016 [39].

However, the evaluation of "tumour budding" is still not standardised [46]. There are many questions regarding the staining technique (haematoxylin-eosin staining (HE) or cytokeratin immunoreaction), the magnification – and therefore the size – of the area to be examined (200x or 400x), and the measurement methodology (presence or extent of tumour budding, examination in one or more fields of view, maximum or average number of tumour cell nests). Despite the methodological problems, a growing number of publications demonstrate that the presence of tumour budding has a negative impact on both OS and DFS in lung squamous cell carcinomas [46].

Minimal cell nest size is defined as the smallest tumour cluster within the tumour or at the invasive front. Minimal cell nest size can be subclassified according to the cell number [30-32; 47; 50]. Weichert et al. reported that in lung squamous cell carcinoma, tumour budding and minimal cell nest size are prognostic markers independent of sex, age and stage [31]. In partial agreement, Kadota et al. demonstrated the adverse prognostic role of the smallest minimal cell

nest size category, single cell invasion and larger cell nuclear diameter [30, 48]. In the assessment of nuclear diameter, the mature lymphocyte nucleus was used as the reference size and tumour cells with a nuclear diameter greater than 4 lymphocytes were defined as having a large nuclear diameter [30; 54; 55].

As demonstrated in our previous publication, the presence of STAS is associated with an unfavourable outcome in lung adenocarcinomas [28; 29] but has been less studied in lung squamous cell carcinomas. The work of Stögbauer and Lu was the first to demonstrate an adverse prognostic impact of STAS in lung squamous cell carcinomas [32; 56].

The grading scheme proposed by Weichert et al. considers tumour budding and minimal cell nest size [31], whereas the grading scheme proposed by Kadota et al. considers tumour budding and nuclear diameter [54]. Although more evidence is accumulating on prognostic markers for lung squamous cell carcinomas, there is still no internationally accepted or recommended grading scheme.

### 1.3. ADJUVANT CHEMOTHERAPY COULD IMPROVE THE SURVIVAL OF PULMONARY SARCOMATOID CARCINOMA: A META-ANALYSIS

Pulmonary sarcomatoid carcinoma has been classified into five subgroups in the latest edition of the WHO classification of Thoracic tumours: pleomorphic carcinoma, spindle cell carcinoma, giant cell carcinoma, carcinosarcoma and pulmonary blastoma [21]. The incidence of sarcomatoid carcinoma is very low, it accounts for less than 1% of lung cancers and 0.1 to 0.4% among NSCLC [58]. At the time of diagnosis, sarcomatoid carcinoma usually has large diameter, aggressive clinical behaviour and high local invasion tendency resulting in poor survival outcome even in patient with early-stage disease [59-61]. Its 5-year OS rate ranges from 12.6 to 34.6% [62; 63]. Complete surgical removal is currently considered to be the best treatment option. Currently, there is no standard treatment for advanced sarcomatoid carcinoma. Specific therapeutic guidelines are not available for sarcomatoid carcinoma; therefore, the NSCLC treatment protocols are generally applied. The benefits of postoperative chemotherapy for OS in patients diagnosed with pulmonary sarcomatoid carcinoma are still controversial. Several studies reported its resistance to chemotherapy [71; 72], while others emphasized the benefits of adjuvant chemotherapy [73-76].

## 2. AIMS

The aims of the present thesis are listed as follows:

2.1 To evaluate semi-quantitatively the extent of STAS and the presence of FTC in lung adenocarcinomas resected by limited (sublobar) surgery; to evaluate their prognostic impact on OS and RFS; and to analyse the reproducibility of assessing these features.

2.2. To investigate semi-quantitatively tumour budding, minimal cell nest size, nuclear diameter, and STAS among patients with resected pulmonary squamous cell carcinoma. Furthermore, we aimed to identify a grading system for the best prognostic stratification for squamous cell carcinoma.

2.3. To investigate the benefit of adjuvant chemotherapy for OS of patients with a diagnosis of pulmonary sarcomatoid carcinoma, to investigate the discrepancies of the management of sarcomatoid carcinoma in published studies and to make recommendations for future research.

## 3. MATERIALS AND METHODS

### 3.1. SEMI-QUANTITATIVE EVALUATION OF SPREAD THROUGH AIR SPACES IN PULMONARY ADENOCARCINOMAS

Patients diagnosed with primary lung adenocarcinoma who underwent sublobar resection of the lung at the Department of Surgery, University of Szeged between 1st January, 2010 and 31st December, 2019 were included. Exclusion criteria were positive surgical margins, perioperative death (within a month of surgery), lack of clinical data and unavailable histological slides. The patients' demographic and clinicopathological parameters including age, gender, smoking history, type of surgery, Eastern Cooperative Oncology Group (ECOG) performance status, adjuvant therapy, histological subtype, IASLC grade [77], necrosis, tumour size, pT and pN status, stage [78], extranodal extension, distance from resection margin, lymphovascular, vascular and pleural invasion were collected from medical records. All patients underwent follow-up that consisted of regular physical examination, chest X-ray, abdominal ultrasonography and chest computer tomography. The follow-up period ended on 1st June 2021.

Formalin-fixed, paraffin-embedded 4-μm-thick sections stained with HE were re-evaluated by two investigators independently (NZT, TZ). During the revision of the sections, the presence of STAS was assessed as follows [21]. The number of tumour cell nests and the presence of

single cell invasion in the alveoli surrounding the invasive tumour were assessed in the three most prominent fields of view at 200x magnification (medium-power field (MPF), field of view = 0.785 $mm^2$). In all cases, the maximum, mean and total number of tumour cell nests were recorded. Based on our preliminary results, we defined the extent of STAS as no STAS (STAS–), low STAS (1-10 tumour cell nests or 1-4 single tumour cells in a 200x field of view) and high STAS (≥11 tumour cell nests or ≥5 single tumour cells in a 200x field of view). In addition, the presence of FTC [23] and the maximum distance of tumour cell nests from the invasive front were assessed during the section revision. Reproducibility of these categories was investigated on digitised slides by four investigators (NZT, SA, AS, TZ) in order to examine inter-observer and inter-method variability.

To decrease bias caused by adverse prognostic variables, two subgroup analyses were done. In the first subgroup, the cases with lymph node metastasis, lymphatic spread, stage III, and pleural invasion were excluded. In the second subgroup, in addition to the former criteria, patients with vascular invasion were omitted, as well.

### 3.2. PROPOSAL OF A GRADING SYSTEM FOR SQUAMOUS CELL CARCINOMA OF THE LUNG

Patients diagnosed with LSCC who underwent surgical resection at the Department of Surgery, University of Szeged between 2010 and 2016 were included. Exclusion criteria were perioperative death, advanced tumours (pT4, distant metastasis), unavailability of histological slides or clinical follow-up data, and neoadjuvant therapy. The patients' clinical parameters including age, gender, smoking habits, type of surgery, adjuvant therapy, and follow-up data, namely OS and RFS were collected from medical charts. All patients had regular follow-up as published and described previously [29]. The follow-up period ended on 1$^{st}$ July, 2022.

Formalin-fixed, paraffin-embedded, HE stained sections were analysed by three independent examiners (NZT, FH, TZ), who were blinded to clinical outcome of the patients. The following morphological parameters were recorded: histological diagnosis defined by WHO [21], tumour size (mm), distance to resection margin (mm), tumour budding, minimal cell nest size, number of mitosis in 10 high-power fields (high-power field (HPF), field of view = 0.237 $mm^2$), nuclear diameter, expansive or infiltrative nature of the invasive front, presence of STAS, vascular-, lymphovascular and pleural invasion. The pT, pN categories, and stages were identified according to the 8$^{th}$ edition of American Joint Committee on Cancer (AJCC) Cancer Staging Manual [78].

Tumour budding was defined as a tumour cell nest with less than 5 cells, surrounded by desmoplastic stroma. Both the presence and the extent of tumour budding were recorded. Regarding the extent, tumour budding was counted with two different methods [54], that is the total number of buds on 10 MPFs and the maximum number of buds in one (hot spot) MPF were registered. The degree of tumour budding was classified according to different cut-off points introduced by Kadota et al. (low and high tumour budding) and Weichert et al. (low, intermediate and high tumour budding) [54; 31].

Minimal cell nest size was subdivided into four categories namely large nest (≥15 tumour cells), intermediate nest (5-14 tumour cells), small nest (2-4 tumour cells), and single cell invasion [30-32; 47; 50]. Minimal cell nest size was recorded at the edge of the tumour and in the entire tumour area.

The nuclear features, such as nuclear diameter (small and large nuclear diameter) and mitotic activity (low and high mitotic rate), were evaluated under HPF [30; 80; 81]. STAS was identified if rounded tumour cell nests were present either in the intra-alveolar space or in the bronchiolar system. Desquamated ribbons of neoplastic cells or tumour cell nests with jagged edges were defined as artefacts and were excluded from investigation.

For pulmonary squamous cell carcinomas, two groups of researchers have recommended a grading scheme. Weichert et al.'s grading scheme focuses on tumour budding and minimal cell nest size [31], while the grading scheme proposed by Kadota et al. considers tumour budding and nuclear diameter [54]. Based on our preliminary results, the aforementioned grading schemes could not significantly separate the three grade groups from each other. Since in our study, the receiver operating characteristics (ROC) curve showed that the combination of tumour budding, single cell invasion and nuclear diameter was the most sensitive and specific predictor of mortality and recurrence ($AUC_{OS}$: 0.83, $AUC_{RFS}$: 0.76); therefore, a new grading system was constructed considering the presence of tumour budding (0-2 points), single cell invasion (0-1 points) and large nuclear diameter (0-1 points). Cases were classified as low (0 points), medium (1-2 points) and high grade (3-4 points). *Table 1* displays the parameters of the proposed grading system.

| Extent of tumour budding | | Single cell invasion | | Nuclear diameter | | Cumulative points | Grade |
|---|---|---|---|---|---|---|---|
| 0 point | 0 bud / 10 MPFs | 0 point | Absent | 0 point | Small | 0 point | Low |
| 1 point | 1-14 bud(s) / 10 MPFs | 1 point | Present | 1 point | Large (> 4 lymphocytes' nucleus) | 1-2 points | Intermediate |
| 2 points | ≥15 buds / 10 MPFs | | | | | 3-4 points | High |

***Table 1.*** **Grading proposal for lung squamous cell carcinoma (MPF: mediate-power field – 200x)**

## 3.3 ADJUVANT CHEMOTHERAPY COULD IMPROVE THE SURVIVAL OF PULMONARY SARCOMATOID CARCINOMA

This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement. The study protocol was registered in the PROSPERO International Prospective Register of Systematic Reviews (CRD42022306084), and we did not deviate from this protocol.

### 3.3.1. Search strategy

The systematic literature search was completed by two independent review authors (NZT, TZ) in three scientific databases, namely MEDLINE (via PubMed), Embase, Cochrane Central Register of Controlled Trials (CENTRAL) up to 9$^{th}$ September, 2021. The following search key was used in all databases: ("sarcomatoid carcinoma" OR "pleomorphic carcinoma" OR "spindle cell carcinoma" OR "giant cell carcinoma" OR "carcinosarcoma" OR "blastoma") AND (lung OR pulmonary). No filter was applied. Reference lists of the eligible studies and the citing articles (via Google Scholar search engine) were also screened to identify relevant publications.

### 3.3.2. Selection and eligibility criteria

Non-randomized controlled studies were found and eligible for inclusion based on the search strategy. Retrospective cohort studies that compared the outcomes of surgical therapy alone (Intervention) with surgery and adjuvant chemotherapy (Control) in patients with PSC (Population) were eligible for inclusion. After removal of duplicates, titles, abstracts then full texts were screened and selected independently by two researchers based on predefined criteria, and a third investigator (SK) resolved all disagreements.

### 3.3.3. Data extraction

Two independent review authors extracted data from the eligible studies into a standardised data collection form. From the selected studies, the following data were extracted: title, first author, publication year, Digital Object Identifier (DOI), all number of patients in each study, number of patients in surgery alone arm (Intervention), number of patients in surgery and adjuvant chemotherapy arm (Control), gender distribution, age and type of adjuvant chemotherapy regimen. Furthermore, hazard ratios (HRs) with the corresponding 95% confidence intervals (CIs) for OS of both univariate and multivariate analysis were also extracted.

### 3.3.4. Risk of bias assessment

Based on the recommendations of Cochrane Collaborations [83], two independent review authors investigated the quality of the included studies using the Risk Of Bias In Non-Randomized Studies - of Interventions (ROBINS-I) risk of bias assessment tool [84]. Low, moderate and serious overall risk of bias were defined as described by Sterne et al. [84].

### 3.3.5. Certainty of the evidence

Certainty of the evidence was evaluated by two independent investigators with GRADE profiler software (GRADEpro GDT: GRADEpro Guideline Development Tool) [85] based on the approach proposed by the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) Working Group [86].

## 4. RESULTS

### 4.1. SEMI-QUANTITATIVE EVALUATION OF SPREAD THROUGH AIR SPACES IN PULMONARY ADENOCARCINOMAS

Altogether 89 patients were operated on with sublobar resection at the Department of Surgery, University of Szeged between 2010 and 2019. Overall 61 patients who diagnosed with primary pulmonary adenocarcinoma were included. The median follow-up time was 32.9 months (range: 5-131 months). In 25 patients, 12 locoregional and 13 distant recurrences were diagnosed. Altogether, 19 patients died from either progression of lung adenocarcinoma or other causes. The presence of STAS was the highest in the high grade (76.4%) followed by the intermediate grade tumours (23.5%). ROC curve analysis focusing on OS and RFS has

identified that the maximum number of tumour cell clusters per 200x field of view and the maximum number of single tumour cells per 200x field of view have the highest area under the curve (AUC) values ($AUC_{tumour\ cluster}$: 0.734; $AUC_{single\ tumour\ cell}$: 0.813), respectively. Based on these parameters, STAS was categorized into three groups, namely "no STAS" (STAS–), "low STAS" and "high STAS". The Kaplan-Meier analysis demonstrated significant differences between OS and RFS estimates of the different STAS categories. FTC was present in 19.7% of patients and had a significant impact on OS. Based on the Kaplan-Meier analysis, there were significant differences of OS estimates of tumours with STAS+/FTC+ and STAS+/FTC– and STAS– tumours. Furthermore, STAS+ cases where FTC was present had the worst prognosis.

In univariate analysis, higher T and N status, higher clinical stage, presence of lymphovascular and pleural invasion, presence of FTC and "high STAS" were associated with unfavourable OS and RFS estimates. In multivariate analysis of OS estimates, higher T category, presence of lymphovascular invasion and FTC were associated with adverse prognosis. Concerning the multivariate analysis of RFS estimates, the presence of lymphovascular invasion and "high STAS" category had unfavourable impact on prognosis.

Our subgroup analysis, excluding adverse prognostic factors, confirmed the prognostic role of STAS. The presence of STAS had an unfavourable impact on RFS, furthermore the data suggest that the higher the degree of STAS, the less favourable the prognosis.

## 4.2. PROPOSAL OF A GRADING SYSTEM FOR SQUAMOUS CELL CARCINOMA OF THE LUNG

Altogether 912 patients diagnosed with lung cancer were operated on at the Department of Surgery, University of Szeged between 2010 and 2016. Overall 220 patients were included in our study. Tumour budding was associated with infiltrative tumour border ($p$<0.001), smaller minimal cell nest size categories ($p$<0.001), single cell invasion ($p$<0.001), larger nuclear diameter ($p$=0.023), pleural- ($p$=0.021), vascular- ($p$=0.006) and lymphovascular invasion ($p$<0.001). Single cell invasion was related to infiltrative tumour border ($p$<0.001), smaller minimal cell nest size categories ($p$<0.001), vascular ($p$=0.05), and lymphovascular invasion ($p$<0.001). Finally, large nuclear diameter was found to be more frequent in smaller minimal cell nest size categories ($p$=0.035).

Altogether recurrence was detected in 54 patients. Thirty patients (13.6%) died from either progression of LSCC or other causes. The median RFS and OS estimates were 19.3 months

(range: 1.9-127.5 months) and 23.0 months (range: 2.1-73.8 months), respectively. The median follow-up was 81 months (range: 1.9-138 months).

In univariate analysis of OS, presence and higher degree of tumour budding, infiltrative tumour border, single cell invasion, large nuclear diameter, higher Kadota-grade, higher Weichert-grade, presence of STAS, higher pT, pN categories and higher stage were associated with adverse prognosis. In univariate analysis of RFS estimates, infiltrative tumour border, smaller categories of minimal cell nest size, presence of single cell invasion, large nuclear diameter, higher Kadota-grade, higher Weichert-grade, presence of STAS, higher pT, pN categories, and higher stage had an adverse impact on prognosis.

Kadota's and Weichert's grading schemes failed to separate the three prognostic categories. Therefore, we aimed to compose a grade stratifying the patients properly according to the prognosis. Thus, we propose a new grading scheme, based on the previously described results, which considers the tumour budding, single cell invasion and nuclear diameter. Our results demonstrated that the proposed grading system can significantly separate the three grading categories for both OS and RFS. Comparing to grading systems published by Kadota et al. and Weichert et al. with our proposed grading system in ROC curve analysis, the latter one had the highest AUC value. Among the findings, we underline that the proposed grading system and STAS were independent prognostic markers in our cohort.

## 4.3. ADJUVANT CHEMOTHERAPY COULD IMPROVE THE SURVIVAL OF PULMONARY SARCOMATOID CARCINOMA

### 4.3.1. Results of systematic search and selection

The PRISMA flow diagram (*Figure 1*) displays the details of selection process. 6,768 records were identified in the three major scientific databases. After removal of duplicates, screening and evaluation for eligibility, four retrospective studies [87-90] were included in our meta-analysis. Each study investigated both surgery alone and surgery and adjuvant chemotherapy arms in patients having pulmonary sarcomatoid carcinoma. In one study, two cohorts were reported separately; therefore, we handled and analysed them separately [89].

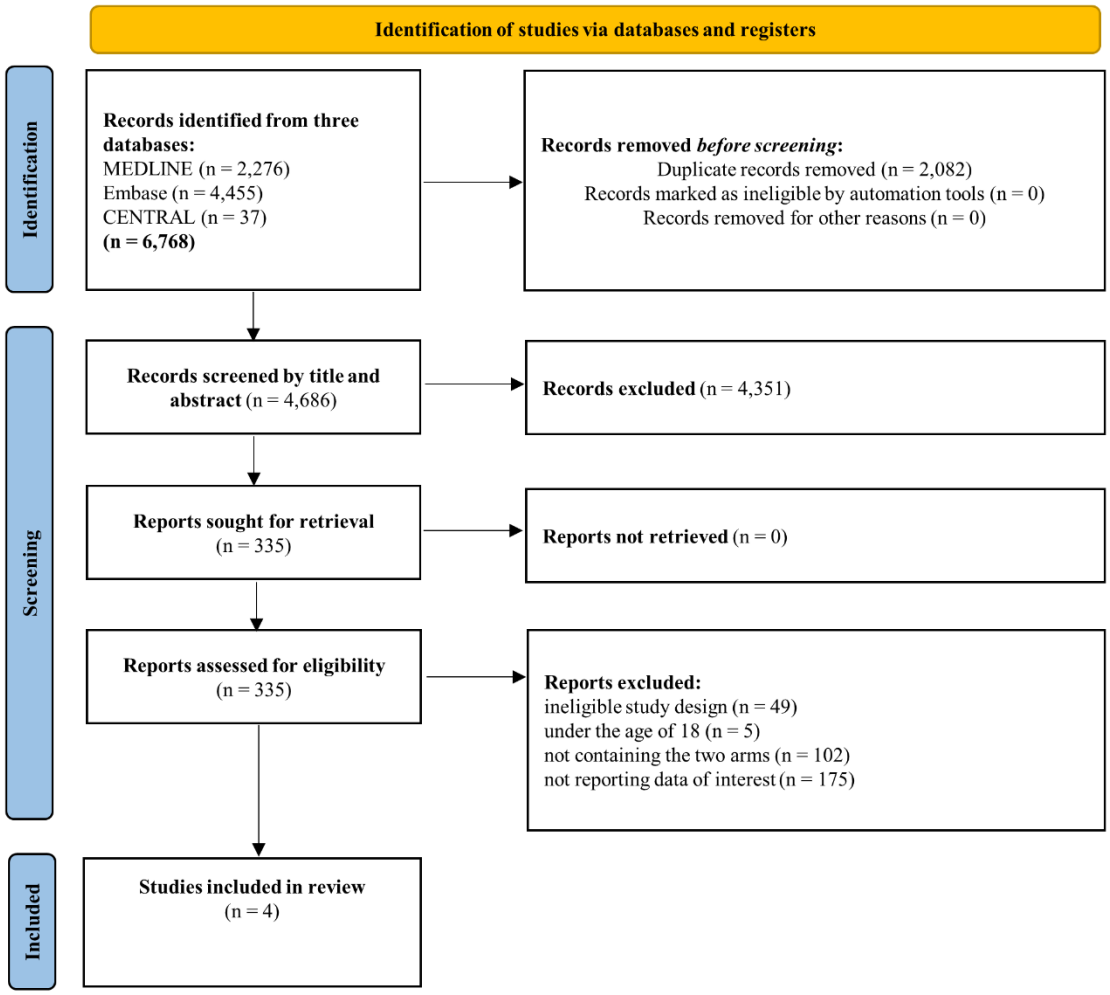


***Figure 1.*** **PRISMA flow diagram (2020) showing details our systematic search and selection process (PRISMA: Preferred Reporting Items for Systematic Reviews, CENTRAL: Cochrane Central Register of Controlled Trials)**

4.3.2. Characteristics of the studies included

From the four articles, altogether 1,852 patients treated with either surgery alone or surgery and adjuvant chemotherapy were included. Diagnosis of PSC was based on resection specimens. Of all participants, only 682 patients' therapeutic regimen was supplemented with adjuvant chemotherapy. From the four articles, only two studies reported precisely the type of adjuvant chemotherapy [88; 90].

4.3.3. Quantitative synthesis of OS

From the 4 articles altogether 5 cohorts (n=1,852) were included for univariate analysis: 1,170 patients underwent surgical treatment only; whereas 682 patients received adjuvant chemotherapy. No statistically significant differences in HR were found between surgery alone

and administration of adjuvant chemotherapy treatment modalities in univariate analysis (HR=0.7138, 95%CI: 0.4989-1.0213, $p$=0.0651).

Altogether three articles reporting on four pulmonary sarcomatoid carcinoma cohorts were included for multivariate analysis [87; 89; 90]. Of all participants, 1,835 patients were included. There was a statistically significant difference between the HR of surgery alone and administration of adjuvant chemotherapy treatment modalities in multivariate analysis (HR=0.5657, 95%CI: 0.4391-0.7290, $p$<0.0001). Based on the recommendations of the Cochrane Collaboration, statistical heterogeneities in both univariate and multivariate analyses were substantial ($I^2_{uni}$=77.7%, $p$<0.01 and $I^2_{multi}$=66.0%, $p$=0.03) [83].

#### 4.3.4. Risk of bias assessment

In both univariate and multivariate analyses, the overall risk of bias for OS was evaluated as moderate. In both analyses, the most common reason for this moderate risk classification was the insufficient description of any analysis for avoiding systematic errors in measurements. Based on the 'bias in selection of the reported result' domain, studies included were sound for a non-randomized study but cannot be considered comparable to a well-performed randomized trial.

#### 4.3.5. Certainty of the evidence

In both univariate and multivariate analysis for OS, certainty of the evidence was 'very low'. The most common reasons of downgrading were the study design and the indirectness (different chemotherapy protocols).

## 5. DISCUSSION

### 5.1. SEMI-QUANTITATIVE EVALUATION OF SPREAD THROUGH AIR SPACES IN PULMONARY ADENOCARCINOMAS

Aerogenic spread, first described by Cain in 1958, is present in primary and secondary neoplasms of the lung; however, this form of tumour spread is more frequent among primary pulmonary adenocarcinomas thereby has been extensively studied [91]. There are some studies which found that in lung adenocarcinoma, presence of STAS was associated with unfavourable prognosis [18; 19; 28; 96]; nevertheless, there are some series, where the prognostic role of STAS could not be supported [97; 98]. Furthermore, according to Blaauwgeers and co-workers claimed the phenomenon called spread through a knife surface (STAKS) [99]. Therefore, the

pathogenesis and the significance of STAS is still debated. However, three meta-analyses have been published recently, in which the presence of STAS was associated with adverse RFS and OS outcome [100-102].

The investigation conducted by Morimoto and associates was the first study focusing on the quantification of STAS and introduced FTC in a rather restrictive morphological definition of the phenomenon. Based on the survival analysis, RFS was significantly reduced in the MPC+/FTC+ group compared to the MPC+/FTC– group [23]. In contrast to the results of Morimoto, we found that FTC is a prognostic factor for OS in univariate (HR: 3.03; 95%CI: 1.09-8.38; *p*=0.034) and multivariate analysis (HR: 5.909; 95%CI: 1.72-20.25; *p*=0.005). Furthermore, we found that FTC+ adenocarcinomas have unfavourable OS and RFS estimates similar to that of pT3 adenocarcinomas (2-year OS rate: 48.2% vs. 46.4%; p=0.16; 2-year RFS rate: 41.2% vs. 35.4%; p=0.18). This finding supports the hypothesis that FTC might rather be an intrapulmonary micrometastasis, which might influence future definitions of the T categories.

Uruga and associates investigated 208 lung adenocarcinomas and classified the extent of STAS into three groups: “no STAS” (STAS–), “low STAS” and “high STAS” based on the number of tumour cell clusters and single tumour cells. They have found that solid subtype of invasive adenocarcinoma, lymphovascular and pleural invasion and ≥10 mm tumor size were related to “high STAS”. Based on univariate and multivariate analysis, “high STAS” was significantly associated with decreased RFS estimates [22]. In our series, the ROC curve analysis has revealed that the maximum number of tumour cell clusters and single tumour cells per 200x field of view have the most acceptable specificity and sensitivity to identify the risk of death and recurrence. The “low STAS” and “high STAS” categories were defined on the basis of the ROC curves. Similarly to their results, we found significant differences among OS and RFS estimates of different STAS categories.

Based on the findings of univariate Cox proportional hazards model, higher pT and pN categories, higher clinical stage, presence of pleural and lymphovascular invasion, “high STAS” category, presence of FTC was associated with unfavourable OS and RFS estimates. Concerning the multivariate analysis, higher T category, presence of lymphovascular invasion and FTC were associated with adverse OS prognosis. According to the findings of multivariate analysis of RFS estimates, the presence of lymphovascular invasion and “high STAS” category had unfavourable impact on prognosis. Our subgroup analysis excluding patients with stage III,

lymph node metastasis, vascular, lymphatic and pleural invasion, demonstrated that “high STAS” category has an adverse impact on RFS estimates. “Low STAS” category was not statistically significantly different from the reference category; however, it showed a trend towards worse outcome. These results are keeping with those of Yang and co-workers. They have presented in their recent meta-analysis that the more extensive the STAS, the more unfavourable the prognosis [101].

Additionally, inter-observer and inter-method variabilities were assessed. On the basis of the intraclass correlation coefficient (ICC) values, the inter-rater agreement was good to excellent for the parameters investigated [79].

The assessment of the extent of STAS is reminiscent of tumour budding in colorectal tumours [22; 38; 39; 51; 54; 103]. We believe that the prognostic role of the extent of STAS deserves further investigation and – in the light of the results – a clinicopathological implementation similar to tumour budding may be expected in the future.

## 5.2. PROPOSAL OF A GRADING SYSTEM FOR SQUAMOUS CELL CARCINOMA OF THE LUNG

Despite the fact that pulmonary squamous cell carcinoma is a frequent primary lung neoplasm, it is not widely investigated. We aimed to analyse the prognostic impact of different morphological characteristics, namely tumour budding, nuclear diameter, minimal cell nest size, and STAS, in a relatively large population of patients diagnosed with resected pulmonary squamous cell carcinoma.

Tumour budding is a morphological pattern of tumour invasion associated with unfavourable prognosis in different carcinomas, namely colorectal adenocarcinoma [34-38], pancreatic adenocarcinoma [40; 41]. Tumour budding has been recently identified as a poor prognostic factor in pulmonary squamous cell carcinoma and adenocarcinoma [30; 49; 106], as well. Furthermore, not only the presence, but the greater extent of tumour budding was associated with adverse prognosis [31; 54]. Kadota et al. and Weichert et al. identified tumour budding categories in 1 HPF and in 10 HPFs with different OS [31; 54] and RFS estimates [54]. Keeping with the aforementioned results, the greater extent of tumour budding was associated with unfavourable OS and RFS estimates in our study. Weichert et al. found that the higher OS estimates were detected in patients with large cell nest size, while decreased OS estimates were associated with single cell invasion [31]. Kadota et al. reported that only the single cell invasion

was an independent prognostic factor [30]. Correspondingly with the results of the aforementioned publications, single cell invasion was proven as an adverse prognosticator for both OS and RFS in our cohort.

Grading systems focusing on nuclear features were established in breast, kidney, bladder carcinoma [107-109] and lung adenocarcinomas [81]; however, its prognostic impact is less evaluated in pulmonary squamous cell carcinoma. Although, nuclear atypia (pleiomorphism) was not statistically significant for predicting prognosis in squamous cell carcinoma, large nuclei were significantly associated with a worse OS estimate [54]. In our cohort, patients with large nuclei were independently associated with worse OS and RFS estimates; however, the mitotic count did not show any association with clinical outcome. The prognostic role of STAS is well investigated in lung adenocarcinomas; furthermore, the presence of STAS in squamous cell carcinoma was associated with unfavourable outcome [56].

Grade is an important prognostic feature of cancers; it influences therapeutic decisions and it is a standard parameter in the stratification of patients for clinical trials [54]. In pulmonary squamous cell carcinoma, Kadota et al. and Weichert et al. have recently proposed grading schemes [54; 31]. As our results demonstrate, both grading systems have significant prognostic roles among patients with squamous cell carcinoma. However, there were no significant differences between Kadota-grade 1 vs. grade 2, and between Weichert-grade 2 vs. grade 3, respectively. Based on our results, tumour budding, single cell invasion, and nuclear diameter have an impact on clinical outcome. Therefore, we propose a grading system which includes these three histomorphological parameters in order to properly identify the prognosis of patients with pulmonary squamous cell carcinoma. In ROC curve analysis, we compared the proposed grading system with the grading schemes published by Kadota et al. [54] and Weichert et al. [31]. According to our results, the proposed grading scheme was superior to others regarding the clinical outcome.

### 5.3. ADJUVANT CHEMOTHERAPY COULD IMPROVE THE SURVIVAL OF PULMONARY SARCOMATOID CARCINOMA

Pulmonary sarcomatoid carcinoma is a low-incidence malignancy that is difficult to study and can only be diagnosed by resection. According to our findings, based on the multivariate analysis, significantly better HRs for OS were found for patients who received adjuvant chemotherapy after surgical therapy. In univariate analysis, there was no significant difference between the HRs for OS of patients with sarcomatoid carcinoma who underwent surgical

therapy alone and those who received adjuvant chemotherapy. The difference between the findings of multivariate and univariate analyses could stem from the covariates used in the multivariate analysis, namely age, gender, race, body mass index (BMI), surgery type (lobectomy and sublobectomy), receiving chemotherapy, grade (grade I-II versus III-IV), histological subtype, tumour size, pathological and clinical stage and nodal metastasis.

In the literature, there are several publications evaluating the different managements of pulmonary sarcomatoid carcinoma and their impact on OS. Systemic chemotherapy alone did not improve survival in patients with PSC. Surgery provides the greatest overall survival benefit and adjuvant chemotherapy may also improve survival. Several studies reported that sarcomatoid carcinoma has more than 70% recurrence rate after surgical therapy, and distant metastases are found more frequently than local metastases [64; 114; 115]. Because of the high recurrence rate and distant metastases, perioperative chemotherapy would have a better efficacy than radiotherapy [87]. Some publications reported beneficial effect of adjuvant chemotherapy on prognosis [74; 75; 87; 89]. These latter results are in keeping with ours. In our meta-analysis, OS was more favourable in patients who underwent surgical resection and received adjuvant chemotherapy. Further investigations are required to evaluate outcomes based on different stages and chemotherapy protocols. Because pulmonary sarcomatoid carcinoma is often diagnosed at an advanced stage and frequently leads to relapse, not only systemic chemotherapy but also targeted therapies are crucial to investigate. In a study, genomic alterations namely TP53 gene and KRAS, found more frequent in sarcomatoid carcinomas; furthermore, other potentially targetable genomic alterations, namely those affecting MET, EGFR, BRAF, HER2 and RET were also identified in sarcomatoid carcinomas. They provided that use of comprehensive genomic profiling in clinical practice may provide important treatment options for this rare, but aggressive neoplasm [116]. Sun et al. investigated the efficacy of target therapy in patients diagnosed with unresectable, locally advanced neoplasms. They found similar results of disease control rate after chemotherapy (58.62%) and after targeted therapy (57.14%) [89]. In contrast, Wang et al. reported that in selected patients who received adjuvant chemotherapy combined with targeted therapy, the outcome was more favourable than for surgical treatment alone ($p$=0.02) [90]. According to the immunotherapy, the PD-1/PD-L1 pathway are an emerging treatment for lung cancer. Vieira et al. found that PD-L1 expression was higher in sarcomatoid carcinomas (53%) than in other NSCLC cases (20%) ($p$<0.001); however, PD-L1 expression did not influence the OS in survival analysis [117]. On the contrary, Velcheti et al. evaluated the expression of PD-L1 which was positive in 69.3% of sarcomatoid carcinoma

cases. Furthermore, the extent of PD-L1 expression was higher in sarcomatoid carcinoma patients than in other types of NSCLC ($p$=0.01) [118]. Evaluation of the efficacy of chemotherapy and immune/target therapy requires further investigation.

In the literature, there are several case reports and retrospective studies which evaluate the prognosis of pulmonary sarcomatoid carcinoma based on different treatment modalities. To our knowledge, this is the first meta-analysis which compared the two therapeutic modalities of surgery alone and surgery with adjuvant chemotherapy in patients with pulmonary sarcomatoid carcinoma.

Based on our findings, after surgical resection, which is needed to establish an adequate histological diagnosis, administering chemotherapy is beneficial and recommended. Our analysis suggests that patients diagnosed with pulmonary sarcomatoid carcinoma have a better survival with adjuvant chemotherapy.

## 6. CONCLUSIONS

In our retrospective study, STAS was investigated semi-quantitatively, and more extensive STAS was related to more unfavourable OS and RFS in adenocarcinomas treated with sublobar resection. Our results prove that the extent of STAS deserves further investigation. Besides of extent of STAS, presence of FTC had an adverse impact on OS. In keeping with the WHO classification, the latest lung cancer reporting protocol introduced by the College of American Pathologists recommends the reporting of STAS, as well [54]. Based on the literature [100-102] and our findings, we propose that the extent of STAS is worth to be reported in routine practice to gather more evidence.

We validated the prognostic impact of morphological parameters, namely tumour budding, single cell invasion, nuclear diameter, and STAS in pulmonary squamous cell carcinoma. For the first time, the grading schemes introduced by Weichert et al. [31] and Kadota et al. [54] were validated, as well. We proposed a combined grading system focusing on tumour budding, single cell invasion, and nuclear diameter for having a proper prognostic stratification in squamous cell carcinoma. Further research is required for validation of the proposed grading scheme and gathering more data about prognostic markers of pulmonary squamous cell carcinoma.

The results of our meta-analysis suggest that patients diagnosed with pulmonary sarcomatoid carcinoma who underwent surgical treatment and were administered adjuvant chemotherapy

have a significantly better OS compared to those who did not receive adjuvant chemotherapy. However, more investigations are required with defined chemotherapy regimens, other treatment modalities (immunotherapy and target therapy), different pathological stages, tumour size, nodal status, type of surgery and histological subtype to identify the most optimal treatment modality for this challenging cancer.

## 7. MAJOR NEW FINDINGS

To our knowledge, this is the first study which evaluated semi- quantitatively STAS and FTC in patient who underwent sublobar resection and diagnosed with pulmonary adenocarcinoma. More extensive STAS was associated with more adverse OS and RFS; furthermore, presence of FTC had an impact on unfavourable OS. In subgroup analysis, STAS was proven to be a significant indicator to determine the prognosis among patient with sublobar resection.

However, there are some investigations of prognostic factors of pulmonary squamous cell carcinoma, there is no internationally accepted grading system for this neoplasm. Based on our research, extent of tumour budding, extent of nuclear diameter and presence of single cell invasion had an adverse impact on OS and RFS. We proposed a combined grading system focusing on the aforementioned morphological parameters for having a proper prognostic stratification among patient diagnosed with pulmonary squamous cell carcinoma.

There is still no consensus on administration of adjuvant chemotherapy in pulmonary sarcomatoid carcinoma. In our meta-analysis, patient who diagnosed with this neoplasm and treated with adjuvant chemotherapy had a favourable prognosis compared to those who were not administered adjuvant chemotherapy.

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LIST OF FULL PAPERS THAT SERVED AS THE BASIS OF THE PH.D. THESIS

I. Zombori-Tóth N, Paróczai D, Lantos J, Almási S, Sejben A, Tiszlavicz L, Cserni G, Furák J, Zombori T. The More Extensive the Spread through Air Spaces, the Worse the Prognosis Is: Semi-Quantitative Evaluation of Spread through Air Spaces in Pulmonary Adenocarcinomas, Pathobiology 2022; 10:1-10.

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