Summary

- **Introduction**

Pulmonary sarcomatoid carcinoma (PSC) is a heterogeneous group of rare epithelial malignant tumours, which are histologically characterized by the presence of a component resembling malignant stromal neoplasms - sarcomas. This group includes, among others: pleomorphic carcinoma, spindle cell carcinoma and giant cell carcinoma.

Pleomorphic carcinomas constitute approx. 0.1-0.75% of non-small cell lung cancers (NSCLC) and approx. 1.5-3% of operated cases. They occur mainly in the sixth decade of life (32-89 years of age), are more common in men, and are strongly associated with cigarette smoking. Clinical and radiological symptoms are similar to those in other lung cancers. Compared to other forms of non-small cell lung cancer, PCs are usually diagnosed at a later stage, have a more aggressive clinical course and a shorter survival, also among operated patients. They also have a worse response to standard chemotherapy and targeted therapy.

- **Assumptions**

Due to the rarity of pleomorphic carcinoma, the data concerning the clinical data, morphology and prognostic factors vary. Most of the studies conducted so far have been based on small groups of patients, up to several dozen people, which makes data analysis, inference and development of a therapeutic strategy for this group of patients much more difficult. There is also no collective data on the Polish population.

Histopathological diagnosis of PC is based on the morphological features of the tumour and does not always require immunohistochemistry. However, it may be helpful in determining morphological subtypes, which may affect the prognosis, further diagnostic management of the material, and, consequently, therapeutic decisions. Literature data on this subject are very diverse.

It is believed that epithelial-mesenchymal transformation processes play a key role in the formation of pleomorphic carcinomas, which is manifested by changes in the expression of substances called EMT markers such as E-cadherin, vimentin, and ZEB1. A gradual loss of E-cadherin is related to the increasing expression of ZEB1 and vimentin, which was observed in molecular studies in pleomorphic carcinomas of other organs and only in single studies on lung pleomorphic carcinoma. It is unclear whether the expression of these markers is related to clinical features and prognosis.

Classic therapeutic strategies currently undertaken in patients with pleomorphic carcinoma, i.e. standard chemotherapy and radiotherapy, as well as molecularly targeted therapy, do not bring satisfactory results. Immunotherapy based on drugs blocking negative immune checkpoints seems to be a promising treatment method due to the high expression of PD-L1 protein on PC cells. High PD-L1

expression may be related to epithelial-mesenchymal transformation, a type of epithelial differentiation and more aggressive course of the disease, but the results presented in the literature are varied.

In recent years, attention has been paid to the negative impact that the spread of cancer through the air spaces (STAS) has on the course of the disease. So far, one study has been published on STAS in pleomorphic carcinoma, showing its negative impact on the prognosis (64). The study group included 35 cases with the accompanying differentiated epithelial component. The relationship between STAS and the type and extent of the mesenchymal component, the expression of epithelial or stromal markers, as well as the expression of the PD-L1 protein has not been analysed.

- **The objectives**

The aim of the study is a retrospective analysis of a group of patients diagnosed with pleomorphic carcinoma, spindle cell carcinoma and giant cell carcinoma of the lung in the surgical material, correlation of morphological features, immunophenotype with clinical data, and identification of factors important in planning further treatment of the patient and predicting the course of the disease and prognosis.

Therefore, the work contains:

1. Morphological analysis of pleomorphic carcinoma, spindle cell carcinoma and giant cell carcinomas, determination of histological features: the type and extend of epithelial and sarcomatoid components, assessment of the extent of necrosis, the severity of inflammatory infiltration, the presence of blood and lymphatic vessels invasion and pleural infiltration.
2. Assessment of the immunophenotype based on immunohistochemical reactions: AE1/AE3, TTF1, p40, E-cadherin, vimentin and ZEB1.
3. Assessment of PD-L1 protein expression in epithelial and sarcomatoid components, correlation with histological type, immunophenotype and expression of E-cadherin, vimentin and ZEB1.
4. Spread through air spaces (STAS) analysis, correlation with PD-L1 protein expression and E-cadherin, vimentin and ZEB1 expression.
5. Determination of prognostic and predictive factors.

- **Material and methods**

The retrospective study covered patients operated on at the Institute of Tuberculosis and Lung Diseases due to lung cancer in 2000-2019 and diagnosed at the Institute's Department of Pathomorphology. The group selected for the study consisted of 107 patients: 100 cases of pleomorphic carcinoma, 5 cases of giant cell carcinoma and 2 cases of spindle cell carcinoma which due to their morphological similarity and clinical features, are considered in the presented study together as a group of pleomorphic carcinoma (PC), in accordance with the latest WHO classification.

Clinical data was obtained from the IT database of the Institute of Tuberculosis and Lung Diseases. Dates of death were completed on the basis of information received from the Universal Electronic System

for Registration of the Population. Additional information was obtained from the National Lung Cancer Register (KRRP) and the National Cancer Register. Overall survival (OS) and disease-free survival (DFS) were used as clinical indicators.

The material was reclassified according to the 2015 WHO classification of lung, pleura, thymus and heart cancers, and the stage of the disease was assessed according to the current TNM classification of 2017. (8th edition). The microscopic evaluation criteria did not differ from those included in the current classification of chest tumours from 2021. In each case, a panel of immunohistochemical antibodies was used, including: cytokeratin AE1/AE3, E-cadherin, vimentin, TTF1, p40, ZEB1 and PD-L1 (clone 22C3). Additionally, the following factors were assessed: the type of epithelial and sarcomatoid pattern with an estimate of their extent within the cancer, the presence of blood (BVI) and lymphatic vessels (LVI) invasion, the extent of necrosis areas, pleural infiltration, inflammatory infiltrates and their intensity. The occurrence of STAS was examined in 99 cases, using the WHO definition and additionally with the semi-quantitative method proposed by Uruga et al. (59).

- **Results**

1. The incidence of pleomorphic carcinoma, based on data obtained from the KRRP in 2000-2019, was 1.6% in the operating material.
2. Pleomorphic carcinoma was more common in men (67M/40F), in the elderly (median 65) and in smokers (92.6%). It was most often localized peripherally (78.5%) and in the upper lobes (51.4%). The median diameter of the tumour was 5.5 cm. Most of the patients were with stage II and III (30.8% and 51.4%). The most common surgical procedure was lobectomy (87%), and only one patient underwent tumourectomy. 90% of the procedures were radical. The clinical course data were available for 70 patients; 26 relapsed (3 local recurrence, 15 distant metastases, 3 metastases and recurrence). Relapse occurred on average after 2.6 years (SD = 2.21), and metastasis after 1.24 years (SD = 1.63).
3. The squamous cell carcinoma component was found in about 46% of cases, adenocarcinoma in about 35%, large cell carcinoma in about 12% and mixed type (glandular-squamous cells) in about 7%. In 22 cases (20.5%), no differentiated epithelial component was found. This group included 5 cases of giant cell carcinoma and 2 cases of spindle cell carcinoma. The epithelial component comprised 1 to 90% of the tumour, on average approx. 36% (median 30), in approx. 18% it was visible only focally and accounted for <10%. The sarcomatoid component in most cases (approx. 56%) consisted of a mixed spindle and giant cells. The spindle cell pattern was most commonly associated with squamous cell carcinoma. Moreover, no clear relationship between the histological type of cancer and the type of the sarcomatoid component was observed. Due to the morphological similarity of cells and their diversity, distinguishing individual epithelial and sarcomatoid components and determining their

quantitative ratio was in many cases very difficult. A univariate statistical analysis showed that the presence of mixed glandular and squamous cell differentiation was associated with more frequent invasion of lymphatic vessels and shorter OS. It was also an independent risk factor for shorter OS. In contrast, squamous cell tissue was one of the independent factors that decreased the risk of recurrence or death. A trend towards a worse median overall survival was also observed in large cell carcinoma and in tumours with giant cell component.

4. Necrosis was present in 90% of cases, in approx. 74% it covered more than 1/4 of the area of the sections. The intensity of necrosis was significantly related to the pT and clinical stage of advancement. Extensive necrosis> 25% was one of the independent factors of shorter DFS.
5. Blood vessels invasion was a more frequent phenomenon (about 73%) than lymphatic vessels invasion (48%). Statistical analysis showed that the invasion of lymphatic vessels was significantly more frequent in tumours with a mixed (glandular-squamous cell) epithelial component and less frequent in cases with large cell carcinoma. In the group with blood vessels invasion, the tumour diameter was significantly larger, as was the pT stage.
6. Various expressions of epithelial and mesenchymal markers were observed in immunohistochemical studies. In 3 cases, no expression of any epithelial markers was found, but only the expression of mesenchymal markers. Epithelial markers (AE1/AE3 and E-cadherin) were more frequent and more intense in the epithelial component than in the sarcomatoid component, while E-cadherin expression was clearly weaker and less frequent in the sarcomatoid component than AE1/AE3. Expression of TTF1 and p40 was found in both the epithelial and sarcomatoid parts in a similar number of cases. The reaction with the anti-vimentin antibody was positive in the epithelial component in approximately 58% of the cases with the presence of this component, and negative in the sarcomatoid component in 15 cases. In these cases, negative expression of ZEB1 and increased expression of epithelial markers in both components were also found, which indicates the lack of activation of EMT processes in these tumours.

   A positive reaction with the ZEB1 antibody was observed in the sarcomatoid part in about 55% of tumours, and in the epithelial component in 3 cases (weak reaction). Positive expression of ZEB1 was correlated with a decrease in E-cadherin expression. The expression of the markers of epithelial-mesenchymal transformation: vimentin, ZEB1 and E-cadherin did not have a significant connection with other parameters, OS and DFS.
7. A positive reaction with PD-L1 was observed in about 64% of cases, in about 42% of tumours the reaction was strong (TPS≥50%). The percentage of positive reactions was higher (approx. 73%) among patients operated on in the period 2010-2019 than in the entire study group ($p = 0.056$). No significant differences were observed in PD-L1 expression in the epithelial and sarcomatoid

components. The most common positive and strong expression of PD-L1 was found in tumours with a large cell carcinoma, and the least frequent in tumours with mixed glandular-squamous cell differentiation ($p<0.05$).

A positive reaction was observed significantly more often in adenocarcinoma than in squamous cell carcinoma. Strong expression was more common in cases with extensive necrosis ≥ 50% and with intense inflammatory infiltration within the tumour ($p<0.05$). There was no correlation between PD-L1 expression and the expression of EMT markers, STAS, OS and DFS.

8. Spread through air spaces (STAS) was found in approximately 39% (18 women and 21 men), slightly more often in adenocarcinomas and mixed carcinomas than in squamous cell carcinoma, but these differences were not statistically significant. STAS was significantly more frequent in patients with a higher stage of the disease, but no significant relationship with other parameters studied was found. Various morphological types of cancer foci in air spaces were found, which, depending on the number, were classified as STAS with low - ls (56.4%) and high - hs (43.6%) severity. Median disease-free and overall survival for the ls and hs groups did not differ significantly, but there were differences in the median OS and DFS between the hs and STAS(-) groups. In the univariate analysis, the presence of STAS and hs was significantly related to the shorter disease-free survival ($p=0.01$ and $p=0.006$), but in the multivariate analysis, the prognostic value of STAS for DFS was variable depending on the criteria adopted in the analysis. The median OS of STAS(+) patients was lower than in the STAS(-) group, but the difference was not statistically significant ($p = 0.052$). In multivariate analysis, the presence of STAS had no effect on OS.

9. Overall survival (OS) data were available in all patients, including 36 patients alive at the end of the study (June 30, 2020). The mean follow-up time was 7.43 years (SD = 4.77) and ranged from 0.56 to 16.95 years. The median OS in the study group was 1.92 years, and overall survival rates at 1, 3 and 5 years were 67.99%, 46.22%, and 41.62%, respectively.

   There were no significant differences in overall survival for men and women ($p = 0.805$). In the univariate analysis, overall survival was statistically significantly related to the stage ($p = 0.014$), lymphatic and ($p = 0.028$) blood vessels invasion ($p = 0.048$), type of epithelial component (the longest median survival for adenocarcinomas and the shortest for mixed cancers $p = 0.006$). There were differences in the median overall survival between tumours with small (up to 25% area) and extensive necrosis (8.3 vs 1.55 years), in the group with giant cell component and other sarcomatoid pattern (0.52 vs 2.79 years), in the STAS(+) group in relation to the STAS(-) group (1.43 vs 3.12 years), and in the hs group in relation to the STAS(-) group (1.19 vs 3.12 years), but these differences were not statistically significant ($p>0.05$).

There was no connection between OS with the expression of mesenchymal markers, the extent of the sarcomatoid component, PD-L1 expression.

Multivariate analysis showed that stage III/IV and mixed type of epithelial component (i.e. adeno-squamous carcinoma or collision adenocarcinoma and squamous cell carcinoma) were significant ($p<0.05$) independent predictors of overall survival.

10. Disease-free survival analysis (DFS) included 70 patients. 24 patients from this group were alive at the end of the study (June 30, 2020), the mean follow-up time in this group was 7.77 years (SD = 4.99) and ranged from 0.56 to 15.64 years. The median DFS was 1.54 years, the disease-free survival at 1, 3, and 5 years was 63.90%, 44.12%, and 35.75%, respectively. There were no differences in DFS for men and women ($p = 0.934$).

    In the univariate analysis, disease-free survival was statistically significantly associated with: clinical stage ($p = 0.011$), severity of necrosis ($p = 0.004$), lymphatic vessels invasion ($p = 0.036$), presence of STAS ($p = 0.01$) and the presence of multiple STAS foci (high STAS) ($p = 0.006$).

    There were differences in median DFS between patients with large cell carcinoma and squamous cell carcinoma (0.35 vs 3.98 years), with giant cell as compared to spindle cell component (0.64 vs 3.98 years) and the group of tumours with and without epithelial component (4.68 vs 1.17 years), but the differences were not statistically significant ($p>0.05$). There were no significant differences in disease-free survival depending on the extent of the sarcomatoid component, expression of mesenchymal markers, expression and intensity of PD-L1, and between the low and high STAS group.

    Multivariate analysis showed that significant ($p<0.05$) independent predictors of DFS are: necrosis >25%, type of epithelial component, and lack of vimentin expression in the sarcomatoid component.

- **Conclusions**

The conducted research allowed the following conclusions to be drawn:

1. Epidemiological data, localization and clinical picture of pleomorphic carcinoma are similar to data in the literature, and the incidence of this form of lung cancer in the Polish surgical material is 1.6%.
2. Determination of the morphological forms of the differentiation, epithelial in particular, has a prognostic significance: cancers with mixed glandular-squamous cell differentiation have worse OS and are more often accompanied by invasion of lymph vessels ($p<0.05$), cancers with squamous cell carcinoma have a better DFS ($p<0,05$). Large cell carcinoma and tumour with giant cell component tend to have worse OS and DFS ($p>0.05$).
3. The extent of each type of histological component is not clinically significant.
4. Immunohistochemical tests are very helpful in PC diagnosis. Reactions with the cytokeratin AE1/AE3 and E-cadherin allow the identification of an often very sparse epithelial component. The reactions with TTF1 and p40 help to define histological differentiation, but these reactions may be expressed

differently within the same tumour. A diffuse cytoplasmic reaction with vimentin in numerous cells helps to distinguish PC from other forms of NSCLC with pleomorphic cells, which may be crucial in the diagnosis of preoperative materials.

5. In the studied group of pleomorphic carcinoma , unfavourable prognostic features include: necrosis (74%), invasion of blood vessels (73%) and lymphatic vessels (48%).
6. The extent of necrosis is significantly related to the degree of clinical and pathological advancement ($p<0.05$). Necrosis >25% is an independent predictor of recurrence or death. Extensive > 50% necrosis is often accompanied by high PD-L1 expression ($p<0.05$).
7. Expression of markers of epithelial-mesenchymal transformation - vimentin and ZEB1- in pleomorphic carcinoma is high, with decreased expression of E-cadherin, which confirms the activation and important role of EMT processes in the development of this tumour. Expression of EMT markers seems to be mainly of diagnostic importance. It has not been shown to be of prognostic significance, or to be connected with STAS and PD-L1 expression.
8. There is a group of cancers with pleomorphic elements, with a differentiated epithelial component, which do not express EMT markers and are associated with a shorter DFS. It requires further research to determine its clinical relevance and classification.
9. In pleomorphic carcinoma, a high frequency (64%) and intensity of PD-L1 expression (TPS≥50%-42%) are observed, which is an important predictor.
10. PD-L1 expression is not related to OS and DFS, but patients with necrosis >25% and positive PD-L1 expression have the lowest median DFS ($p = 0.023$) and should be systematically followed up to detect relapse early.
11. The STAS found in 39% of the examined tumours, was more frequent in patients with a higher stage of the disease ($p<0.05$). There was no correlation between the presence of STAS and vascular invasion, the extent of necrosis, the histological type of differentiation, or the extent of the sarcomatoid component. There was also no association with the expression of EMT markers.
12. The quantitative criteria for STAS assessment proposed by Uruga et al. - low and high STAS - are a promising direction in the development of research on the prognostic significance of STAS.
13. STAS is prognostic for DFS: patients with STAS have worse DFS in univariate analysis, especially patients with more STAS lesions ($p<0.05$). In multivariate analysis, the prognostic value of STAS for disease recurrence is variable, indicating that it is an important prognostic factor, but in combination with other parameters. Patients with STAS tend to have poorer overall survival ($p>0.05$).
14. The prognostic factors for OS are as follows:
    - in univariate analysis: stage III/IV ($p = 0.014$), lymphatic vessels invasion ($p = 0.028$) and blood vessels invasion ($p = 0.048$), mixed glandular and squamous epithelial component ($p = 0.006$);

- in multivariate analysis, independent predictors are: stage III/IV (p = 0.037) and mixed glandular and squamous epithelial component (p = 0.003).

15. The prognostic factors for DFS are:
    - in univariate analysis: stage III/IV (p = 0.011), severity of necrosis (p = 0.004), lymphatic vessels invasion (p = 0.036), presence of STAS (p = 0.01) and the presence of multiple STAS foci (high STAS) (p = 0.006);
    - in multivariate analysis, the independent predictors are: necrosis > 25% (p = 0.015), type of epithelial component - squamous cell structure has better prognosis (p = 0.013) and no vimentin expression in the sarcomatoid component (p = 0.022).

The presented analysis is retrospective in nature, which points to a certain constraint of the study. It should be emphasized, however, that this is - so far - the only extensive study of pleomorphic carcinomas in Poland, additionally covering the cases from only one centre.