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ORIGINAL ARTICLE

Spread through air spaces (STAS) is a predictor of poor outcome in atypical carcinoids of the lung

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Abstract

Spread through air spaces (STAS) have been recently recognized as a prognostic factor for adenocarcinoma and squamous cell carcinoma of the lung. Pulmonary neuroendocrine neoplasms (NENs) include tumors with different morphology and a heterogeneous clinical behavior. Among atypical carcinoids (ACs), new prognostic factors able to refine prognosis are needed. In the present study, a retrospective series of 91 surgically resected ACs was investigated, in parallel with 191 control cases of typical carcinoids (TCs) and of high-grade small- and large-cell neuroendocrine carcinomas, to assess the presence and potential prognostic role of STAS. STAS was defined by the presence of neoplastic nests or single cells in air spaces beyond the tumor edge. Clinicopathological parameters and survival were correlated by univariate and multivariate analyses. STAS was identified in 48% of ACs (44/91) compared to 20.5% of TCs and 71–88% of high-grade large- and small-cell carcinomas in the control group. In the carcinoid group, presence of STAS was significantly correlated with unfavorable parameters, such as high tumor stage, positive nodal status, high Ki-67 index, presence of angioinvasion, and with adverse disease outcome, shorter overall survival, and time to progression. In conclusion, the presence of STAS is an additional relevant adverse prognostic factor in pulmonary AC that currently has the most unpredictable outcome and the most controversial treatment strategy.

Keywords Lung · Atypical carcinoid · Neuroendocrine neoplasm · Spread through air spaces (STAS) · Prognosis

Serdar Altinay and Jasna Metovic contributed equally to this work.

ETHICAL RESPONSIBILITIES OF AUTHORS
All individuals listed as co-authors of this manuscript qualify for every one of the four following criteria:
- Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
- Drafting the work or revising it critically for important intellectual content; AND
- Final approval of the version to be published; AND
- Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Introduction

In non-small cell lung cancer (NSCLC), novel immunophenotypic or molecular markers have recently emerged with relevant prognostic and predictive impact, but also previously underestimated morphological parameters have been recognized to impact prognosis. These include refined staging criteria [1] as well as distance of the tumor from resection margins, tumor budding, single cell invasion in the tumor periphery, and spread through air spaces (STAS) [2–5].

In particular, STAS is a morphological feature that seems to be easily assessed at the pulmonary tumor border of conventional hematoxylin and eosin (H&E)-stained slides, being related to the presence of floating tumor cells, arranged into small or large neoplastic clusters in the peritumoral alveolar spaces of the lung. Similarly to "tumor budding," which is evaluated within the peripheral fibrous tissue of the invasive front of different cancers (e.g., colorectal carcinoma and lung carcinoma), STAS represents another form of tumor spread that characterizes the invasive properties of malignant pulmonary neoplasms. Its presence was extensively investigated in pulmonary adenocarcinoma [3] and squamous cell carcinoma [4] and found to be an independent predictor of recurrence and fatal outcome in these histotypes. STAS was recently reported at high frequency in resected small cell lung carcinoma, but no prognostic correlation, either with disease free or overall survival (OS), was identified [5].

Carcinoid tumors have not been investigated so far, with the exclusion of a marginal mention in the context of a case reported by Masai et al. [6]. In another study [7], infiltrative growth ("of the adjacent normal architecture," as described by the authors) was investigated in carcinoid tumors. However, no specific mention was made to infiltration through air spaces, and in any case, no significant differences were identified between typical and atypical carcinoids (TC and AC), with no mention to any correlation of this finding with outcome.

Apart from criteria included in the current WHO classification of carcinoids [8, 9] (namely mitotic index and necrosis), factors predisposing to poor prognosis are uncertain, with special reference to AC that generally show a more aggressive behavior and lymph node involvement in 20% to 60% of cases [10–12].

In lung carcinoid tumors, Ki67 only partially complements mitotic count, and different values of proliferation have been identified in both typical and atypical forms, leading to the proposal of a grading system combining the two classical morphological parameters with Ki67 index [13–15].

The aim of this retrospective study was to explore the prevalence of STAS in AC tumors, as compared to a control group of TCs on the one side and of high-grade neuroendocrine large- and small-cell carcinomas on the other, and to correlate its presence with clinical-pathological parameters and disease outcome. We here demonstrate that presence of STAS is a strong independent negative prognostic indicator in ACs.

Materials and methods

Case selection

A retrospective series of 91 surgically resected lung ACs was retrieved from the pathology files of the University Hospitals "Città della Salute e della Scienza" in Turin and "San Luigi" in Orbassano in a period from 1989 to 2015. Inclusion criteria were availability of original H&E slides and/or of paraffin blocks for preparing freshly cut H&E sections, as well as follow up information. A control series of 191 surgically resected pulmonary NENs, including 161 TCs and 30 high-grade large- and small-cell neuroendocrine carcinomas, was also collected from the same institutions in the same time period. All original H&E slides were reviewed by three of the authors (SA, MV, MP) to confirm the diagnostic categorization according to the WHO criteria [8]. Some of the considered cases had already been investigated in a previous study assessing the role of Ki-67 in pulmonary carcinoids [15]. An ad hoc database was generated, and clinical-pathological features and follow-up data were annotated. The study was approved by the local ethical committee (Department of Oncology at San Luigi Hospital, number 17975/2015). All cases were anonymized by a pathology staff member not involved in this project, and statistical and correlative analyses were performed using coded data, only.

Microscopic assessment of STAS

STAS was independently investigated by the same three authors (SA, MV, MP) who were blinded to the patients' outcome at the time of microscopic revision. In case of diagnostic discrepancies, a consensus was reached discussing each case at a multiheaded microscope. Tumor STAS was evaluated according to the original description and the WHO definition as floating tumor nests or single cells in the air spaces beyond the outer border of the neoplastic mass [3, 4, 8]. This can occur to a variable extent in the first alveoli adjacent to the tumor edge or in spaces further away. At low magnification, the presence of STAS was relatively easy to identify due to the generally well-demarcated borders of carcinoid tumors. STAS may present as either large tumor nests or as a single-cell pattern, with few intra-alveolar free-floating tumor cells [4, 5]. The above-described features of STAS were carefully distinguished from potential mimickers or artifactual conditions. The former includes reactive or atypical pneumocytes which generally line alveolar spaces rather than being free floating, and in any case are less atypical. Similarly, alveolar histiocytes usually have no cytological atypia and contain pigments or

vacuoles. Although tumor budding is not a common feature of carcinoid tumors, it may be relatively easily distinguished from STAS being the neoplastic cells or clusters localized within the stroma at the outer tumor border rather than within the pulmonary alveoli. More importantly, some carcinoids can be associated with other neuroendocrine proliferative lesions, such as tumorlets or neuroendocrine cell hyperplasia, which may develop in the peritumoral pulmonary parenchyma and mimic carcinoid cell spread, although these conditions are more commonly centered around the bronchial tree (rather than the alveoli) [4, 5]. Regarding artifacts, tumor cells may detach during tumor dissection and disseminate in peritumoral air spaces. The correct distinction from real STAS may be difficult and is generally based on the irregular and random distribution of such detached tumor fragments at the edges of the tissue section, rather than around the tumor edge. In addition, in the differential diagnosis, we also found of aid the reported sharpness of some borders of the spreading tumor clusters, as a result of the artifactual dissection, as described by Kadota et al. [3].

Statistical analysis

The presence of STAS was correlated with major clinical and pathological features using Chi-square and Fisher tests or Student's *t* test, as appropriate, either in the entire cohort or in carcinoid patient samples, only, when dealing with parameters associated with prognosis. Status was available for 209 cases. Based on the available clinical data, univariate time to progression (TTP) survival analysis was performed on 114 carcinoid cases and OS analysis on 188 carcinoid cases with the Kaplan-Meier method; the log-rank test was employed to compare survival curves. Cox proportional hazards regression model was used in multivariate survival analysis to assess the independent role of parameters significant at univariate analysis. Univariate analyses of OS and TTP were also performed in AC group (onto 68 and 39 cases, respectively), only. STAS and single-cell invasion was also tested by means of OS analysis in TC group (onto 120 cases), only. TTP survival analysis independently in the TC group was not assessable due to the very limited number of events in this group (1 single case with progression over 75 cases with information). A $p < 0.05$ value was considered statistically significant.

Results

Clinical-pathological features

The 91 AC tumors here investigated had a mean age of 60 years (range 23–83) with a slight male predominance (47/44). The mean tumor size was 3.1 cm (range 0.8–8.5). Necrosis was present in 51/91 cases, and vascular invasion was observed in 48% of cases. High pT stage (pT3 or pT4) was present in 16% of cases, and positive nodal status was recorded in 42% of cases. The mean proliferation index of ACs, as detected by Ki-67 immunostaining, was 9.6%. The mean follow up time was 59 months (range 1–244).

Pattern of STAS and correlation with tumor histotype

STAS was identified in 48% of ACs. Small or large neoplastic clusters were identified in the peritumoral air spaces, as well as a pattern of single-cell invasion in approximately half of positive cases (57%) (Fig. 1). In the control cases, STAS was present in 20.5% of TCs and in 76.7% of the high-grade carcinomas ($p < 0.0001$) (Table 1). STAS generally occurred in peritumoral air spaces closer to the AC edge, the farthest observed case having tumor cell cluster spread up to seven alveolar spaces away from the tumor border (Fig. 1b). In control cases, TCs had similar features as ACs, while in high-grade NE carcinomas, more heterogeneous tumor cell-spreading patterns were observed in the peritumoral parenchyma, at variable distances from the tumor border (Fig. 1c, d). Special attention was paid to distinguish neoplastic cells featuring STAS from artifacts or non-neoplastic cells, such as atypical/reactive alveolar macrophages or detached pneumocytes. In the latter case, chromogranin A immunostaining was useful to solve controversial cases, highlighting even single neoplastic neuroendocrine cell spread in air spaces (Online Resource 1). Artifactual spread of neuroendocrine cells in alveolar spaces during gross tumor dissection was an extremely rare event in ACs. It was identified when neoplastic cells were irregularly located in air spaces even at great distance from tumor edges and when such neoplastic clusters had one or more sharp border. In addition, these cases were not associated to single neuroendocrine cell invasion.

Clinicopathological correlations

In the carcinoid patient group, the presence of STAS was significantly associated with parameters indicative of a more aggressive disease, such as high pT stage, presence of positive lymph nodes, high proliferation index, presence of angioinvasion, and adverse clinical outcome (either alive with or died of their tumor) (Table 1). Moreover, in the AC group, the presence of STAS was associated with presence of necrosis and high mitotic count. The presence of single-cell invasion pattern was significantly associated with all the above, except for pT stage and, in the AC group only, necrosis and mitotic index.

Correlation of STAS with clinical outcome

The impact of STAS and single-cell invasion on prognosis was determined as TTP and OS, in comparison with the most

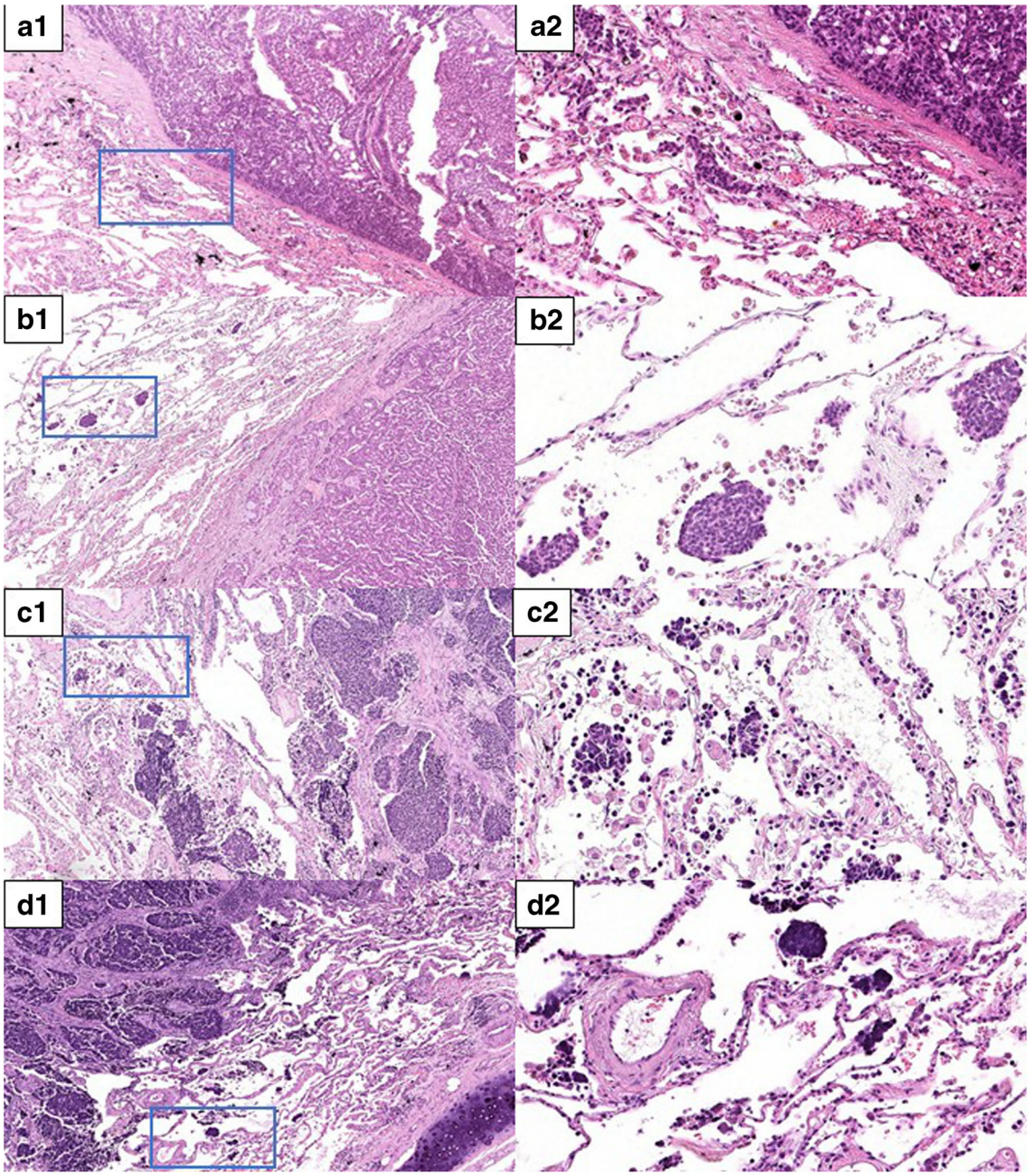

Fig. 1 Spread through air spaces (STAS) in neuroendocrine pulmonary tumors. The presence of tumor cell clusters or single-cell invasion may occur in all histological types, as shown in examples of typical carcinoid (a1/2) and atypical carcinoid (b1/2). The control group of high-grade neuroendocrine carcinomas also demonstrated extensive STAS, even in air spaces far from the tumor edge, as shown in these examples of surgically resected large cell neuroendocrine carcinoma (c1/2) and small-cell carcinoma (d1/2) (H&E; original magnifications: 50× in a1,b1,c1,d1; and 200× in a2,b2,c2,d2)

relevant clinical and pathological parameters in our series of carcinoids. At univariate TTP survival analysis (Table 2), both single-cell invasion pattern and STAS were associated with shorter survival (Fig. 2, left panels), together with male sex, atypical histotype, positive nodal status, Ki-67 above or equal to 4%, and presence of angioinvasion. At multivariate analysis of TTP, male sex, AC histology, and positive nodal status, only, retained statistical significance. However, testing the same parameters at univariate survival analysis in the AC group only, the presence of STAS was the single negative prognostic indicator. Very similar results were observed at OS analysis (Table 3). At univariate analysis, the same parameters associated with prognosis in terms of TTP were associated to a specific OS, including single-cell invasion and STAS (Fig. 2, right panels). Moreover, at multivariate analysis, the presence of STAS retained a statistically significant

Table 1 Association of spread through air spaces (STAS, with tumor cell clusters or single-cell invasion patterns) with relevant clinical and pathological features of 282 surgically resected neuroendocrine neoplasms of the lung

Overall series (no. 282)							
Parameter		STAS − (no. 182)	STAS + (no. 100)	*p*	Single-cell invasion − (no. 228)	Single-cell invasion + (no. 54)	*p*
Sex	M	76	59	0.006	103	32	0.07
	F	106	41		125	22	
Age	median	57	66	< 0.0001	59	66	0.006
Histotype	TC	128	33	< 0.0001 < 0.0001[a]	149	12	< 0.0001 < 0.0001[a]
	AC	47	44		63	28	
	LCNEC	6	15		13	8	
	SCLC	1	8		3	6	
Carcinoid tumors, only (no. 252)							
Parameter		STAS − (no. 175)	STAS + (no. 77)	*p*	Single-cell invasion − (no. 212)	Single-cell invasion + (no.40)	*p*
Size (cm)	mean	2.9	2.6	0.28	2.8	2.9	0.45
pT stage (6 missing)	1–2	157	57	0.0023	182	30	0.07
	3–4	15	17		23	9	
pN stage (8 missing)	0	142	46	0.0014	166	22	0.009
	1–2	29	27		41	15	
Ki-67 (%)	mean	4.0	8.4	0.0003	4.6	9.1	0.0001
Mitotic index (in AC group, only)	mean	2.6	4.3	0.003	3	4.2	0.067
Necrosis (in AC group, only)	no	26	10	0.017	29	7	0.06
	yes	25	30		34	21	
Location (94 missing)	central	59	18	0.08	66	12	1.0
	peripheral	51	29		67	13	
Angioinvasion (7 missing)	no	130	34	< 0.0001	148	16	0.0035
	yes	43	38		61	20	
Status (43 missing)	NED/DOC	135	39	0.0001	156	18	0.011
	AWD/DOD	15	20		25	10	

STAS spread through air spaces, *M* male, *F* female, *TC* typical carcinoid, *AC* atypical carcinoid, *LCNEC* large cell neuroendocrine carcinoma, *SCLC* small cell lung carcinoma, *NED/DOC* no evidence of disease/died of other cause, *AWD/DOD* alive with or died of disease

[a] TC vs AC, only

Table 2 Univariate and multivariate time to progression survival analyses in 114 surgically resected lung carcinoids

	All carcinoids				Atypical carcinoids, only	
	Univariate analysis (TTP)		Multivariate analysis (TTP)		Univariate analysis (TTP)	
Parameter	HR [CI]	*p*	HR [CI]	HR [CI]	HR [CI]	*p*
Male sex	3.15 [1.23–8.07]	0.017	2.19 [0.78 to 3.59	0.0023	2.55 [0.97–6.69]	0.05
Age (below median)	0.56 [0.22–1.43]	0.23	–	–	0.59 [0.23–1.56]	0.29
Atypical histotype	30.31 [10.70–85.86]	< 0.0001	3.25 [1.09 to 5.41]	0.0033	–	–
Size (cm) (below median)	0.77 [0.30–1.95]	0.57	–	–	1.34 [0.48–3.73]	0.57
pT stage 3/4	1.25 [0.32–4.80]	0.74	–	–	0.99 [0.28–3.50]	0.98
Positive nodal status	11.28 [3.62–35.15]	< 0.0001	2.23 [0.63 to 3.83]	0.0065	2.54 [0.94–6.89]	0.06
Ki-67 (≥ 4%)	5.28 [1.87–14.90]	0.0016	0.80 [− 0.59 to 2.20]	0.2645	1.39 [0.52–3.68]	0.50
Presence of angioinvasion	7.47 [2.72–20.50]	< 0.0001	0.20 [− 1.06 to 1.46]	0.7566	2.53 [0.87–7.29]	0.08
Presence of single-cell invasion	6.11 [1.15–32.57]	0.033	−0.65 [− 2.13 to 0.83]	0.3903	0.95 [0.31–2.96]	0.93
Presence of STAS	6.49 [2.15–19.62]	0.0009	0.71 [− 0.61 to 2.04]	0.2945	2.45 [0.91–6.61]	0.049

STAS spread through air spaces, *TTP* time to progression, *HR* hazard ratio, *CI* confidential intervals

association with adverse prognosis together with male sex, atypical histotype, and presence of angioinvasion. To test the combined effect of parameters significant at OS multivariate analysis, we created a model attributing a score to each significant variable according to its HR (e^b). Thus, a score value of 1 was given male gender, presence of STAS, and presence of angioinvasion (score 0 was given in the absence of these parameters), whereas a score of 2 was given to atypical histotype (score 0 if typical histotype), with a range from 0 to 5. Cases were then separated in three groups, including score ≤ 2 (111 cases), 3 (20 cases), and 4–5 (33 cases). Univariate analysis of OS showed a significant increased risk of death on the basis of score value ($p < 0.001$) (Online Resource 2). Finally, presence of STAS was significantly associated to shorter OS in the AC group, together with presence of lymph node metastases. OS analysis was also performed in the TC group, and both STAS and single-cell invasion were significantly associated with shorter survival (Online Resource 3), together with male sex and older age, as for the whole group of carcinoids (Table 3).

Discussion

In this study, we have demonstrated that spread through air spaces (STAS) is a novel adverse prognostic feature in pulmonary ACs, a subgroup on neuroendocrine neoplasms that currently present with a relative unpredictable outcome. STAS was strongly associated with clinical and pathological features of aggressiveness and with TTP and OS. Although STAS proved to be a valuable prognostic factor in NSCLC, both in the adenocarcinoma and squamous cell carcinoma histotypes, it has never been investigated in carcinoid tumors. Indeed, our study demonstrated that STAS is present in a considerable subgroup of lung carcinoids especially among the atypical subtype with associated prognostic value. In this context, we observed that STAS is relatively easy to assess in conventional H&E stained slides and is not a time-consuming procedure. Carcinoid tumors are generally well-demarcated tumors and the peritumoral air spaces are easily investigated, taking only care to exclude potential mimickers of STAS in the peritumoral parenchyma, such as reactive/atypical macrophages, neuroendocrine cell hyperplasia, tumorlets, and finally tumor cell groups artifactually dissected during specimen processing [4, 5].

In the literature, a recent paper demonstrated that STAS was of prognostic value in a large series of resected lung adenocarcinomas. Strict diagnostic criteria for the differential diagnosis between real STAS and possible artifacts were outlined, supporting the concept that STAS is a biological event associated with a higher propensity for invasive behavior rather than a product of tissue manipulation [16]. The distinction of neoplastic spreading cells or clusters from non-tumoral cells is relatively straightforward, despite the low degree of atypia in carcinoids; in difficult cases, immunostaining for neuroendocrine markers (e.g., chromogranin A or synaptophysin), which are routinely available in the diagnostic work up, may highlight the alveolar spread of NE tumor cells. Therefore, this feature can be a useful additional parameter to be incorporated in the diagnostic checklists for lung carcinoid reporting.

According to the literature [5], different patterns of tumor STAS were identified in NSCLC, including large nests, small neoplastic clusters, or single-cell invasion. We followed the proposed recommendations and separately recorded the single-cell invasion pattern, while it was more complicated to consider separately small and large neoplastic cell clusters in the series of carcinoid tumors here investigated. Moreover, very recently, a semiquantitative subclassification of STAS-

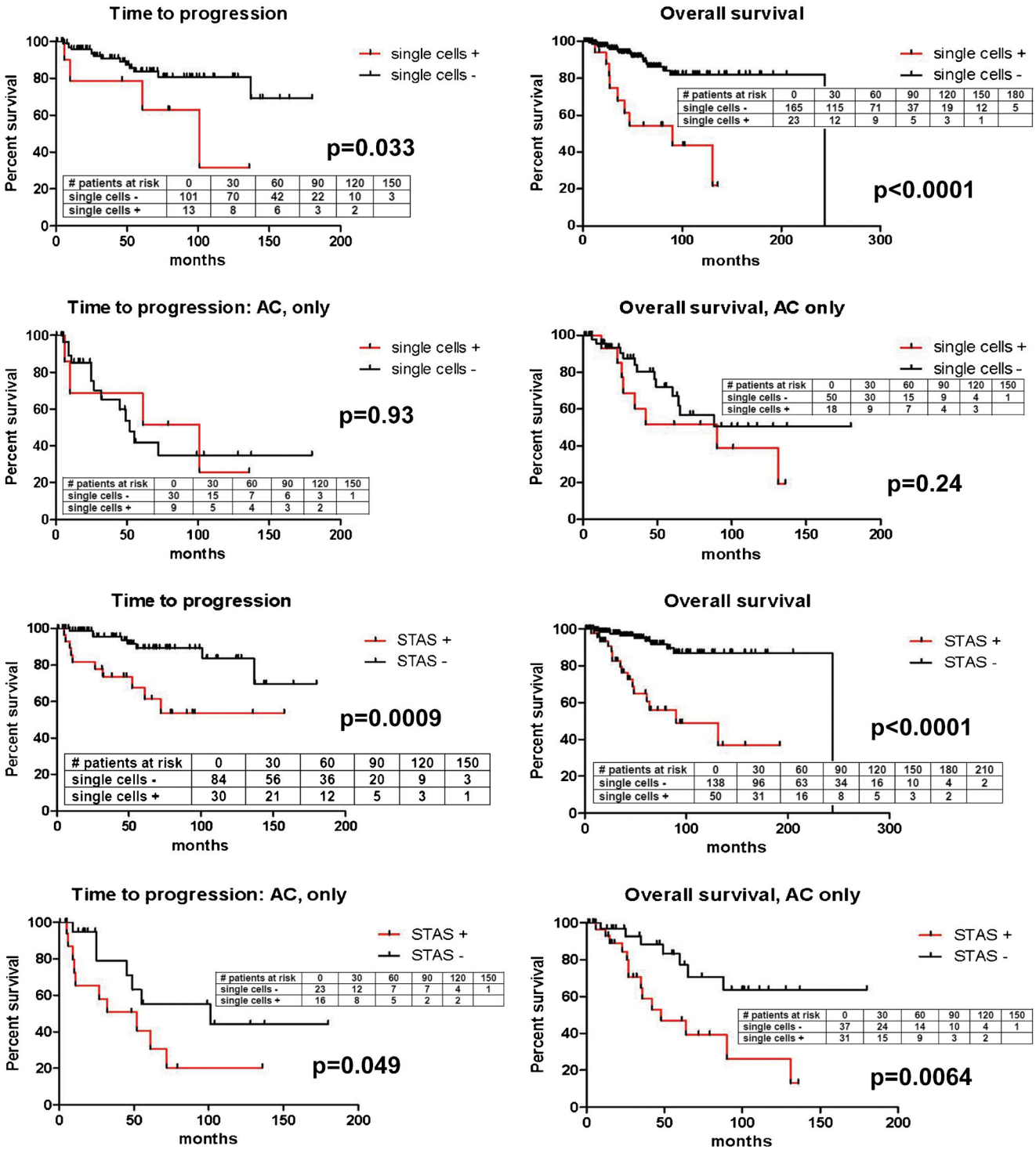

Fig. 2 Kaplan Meier curves of time to progression (TTP) and overall survival (OS) analyses of single-cell pattern and STAS-positive and negative pulmonary carcinoid tumors, as a whole and in atypical carcinoid group, only

positive cases into low and high extent has been proposed in lung adenocarcinoma [17]. However, although we cannot exclude that the extent of STAS and/or single-cell invasion might further influence their prognostic impact, we did not include a substratification of positive cases into low or high positive in our study because there are no validated cut-offs for the definition of STAS extent and to avoid possible bias due to heterogeneous sampling procedures in our retrospective series. Invasion as single cells occurred in parallel to the presence of tumor clusters in approximately half cases. When this combined tumor cell spreading modality occurred, a significant association with angioinvasive properties of the

Table 3 Univariate and multivariate overall survival analyses in 188 surgically resected lung carcinoids

	All carcinoids				Atypical carcinoids, only		Typical carcinoids, only	
	Univariate analysis (OS)		Multivariate analysis (OS)		Univariate analysis (OS)		Univariate analysis (OS)	
Parameter	HR [CI]	*p*	Coefficient [CI]	*p*	HR [CI]	*p*	HR [CI]	*p*
Male sex	3.41 [1.55–7.51]	0.0023	1.48 [0.27 to 2.69]	0.017	2.30 [0.97–5.43]	0.06	9.11 [1.27–65.6]	0.028
Age (below median)	0.40 [0.18–0.87]	0.02	1.48 [− 1.09 to 1.11]	0.98	0.63 [0.26–1.50]	0.30	0.12 [0.12–0.84]	0.033
Atypical histotype	14.34 [6.04–34.02]	< 0.0001	2.39 [0.73 to 4.04]	0.0048	–	–	–	–
Size (cm) (below median)	0.78 [0.39–2.03]	0.89	–	–	0.79 [0.32–1.97]	0.62	1.26 [0.17–9.12]	0.82
pT stage 3/4	1.53 [0.50–4.65]	0.45	–	–	1.56 [0.50–4.87]	0.44	0.31 [0.02–5.02]	0.41
Positive nodal status	8.06 [2.87–27.65]	< 0.0001	0.46 [− 0.83 to 1.77]	0.48	3.65 [1.39–9.59]	0.0086	0.32 [0.02–5.86]	0.44
Ki-67 (≥ 4%)	3.60 [1.55–8.32]	0.0028	0.80 [− 0.94 to 1.22]	0.81	1.98 [0.79–4.94]	0.14	2.02 [0.25–16.06]	0.50
Presence of angioinvasion	10.32 [4.04–26.38]	< 0.0001	1.94 [0.32 to 3.56]	0.019	1.02 [0.32–3.10]	0.99	3.31 [0.14–76.34]	0.45
Presence of single cell invasion	17.69 [4.80–65.21]	< 0.0001	−0.21 [− 0.37 to 0.93]	0.71	1.79 [0.67–4.75]	0.24	754.4 [3.32– > 1000]	0.016
Presence of STAS	11.60 [4.50–29.94]	< 0.0001	0.71 [− 0.11 to 2.73]	0.034	3.46 [1.42–8.46]	0.0064	16.94 [1.06–270.9]	0.045

STAS spread through air spaces, *OS* overall survival, *HR* hazard ratio, *CI* confidential intervals

individual tumors was detected. This is not surprising, if single tumor cells in an alveolar space are considered to reflect the invasive capacity of the tumor, as can occur in the case of blood vessel invasion. The invasive potential of cells spread through air spaces has recently been supported by the recent demonstration of high MET expression in NSCLCs, not only present at the invasive front of the tumor but also within STAS [18]. Furthermore, the occurrence of STAS might promote seeding of tumor cells in the lung parenchyma that in turn could subsequently adhere to the alveolar surface even far from tumor edges, as supported by the major risk of local recurrences in STAS-positive patients affected by stage I adenocarcinoma treated by limited lung resections [3].

Comparing the occurrence of STAS in ACs with that of control groups of TCs and high-grade neuroendocrine carcinoma, it was noticed that STAS increased in the spectrum of NENs, paralleling the reduced differentiation of the different histological types, from TC to small cell carcinomas. With regard to the distribution of STAS, in AC tumors, it was mainly detected in proximal peritumoral alveolar spaces, generally in the first or second layer of air spaces, partially atelectatic or compressed by the tumor mass, with few exceptions. Conversely, in the control series of high-grade large- and small-cell neuroendocrine carcinomas, a heterogeneous distribution of STAS in spaces either nearby or far away from the tumor edge was observed in the majority of cases (76.6%). Therefore, as expected, STAS as either cell clusters or the pattern of single-cell invasion increased along with loss of tumor differentiation. We assume that STAS is a very common feature of high-grade neuroendocrine carcinomas, even more common than that observed in adenocarcinomas or squamous cell carcinomas of the lung (reported in less than 30% of cases) [3–5]. There are no published data on specific subtypes of neuroendocrine carcinomas, since for the 14 large- or small-cell carcinomas included by Masai and coworkers in their study on over 500 lung cancers, no separate data were reported [5]. Further studies specifically designed on high-grade neuroendocrine lung cancers are therefore needed.

In carcinoids, STAS was found to correlate with several pathological parameters of aggressiveness. Although some morphological parameters are effective to stratify NEN subgroups with significantly different outcomes (e.g., TC vs AC vs high-grade NE carcinomas), within the individual groups, especially ACs, the natural history of the disease is relatively heterogeneous and unpredictable. The two acknowledged diagnostic parameters, i.e., mitotic count and necrosis [8], may need to be coupled with other factors. We therefore wondered whether STAS-positive cases in the AC subtype were more commonly associated with necrotic tumors or to a higher mitotic count. Indeed, it seemed that the presence of necrosis was a feature of STAS-positive AC. This observation may suggest the opportunity of formulating a multi-parameter score that combines the official criteria (mitotic index and necrosis) with other relevant factors that may help to better refine the diagnostic and prognostic characterization of carcinoid tumors. These may well include Ki67 index, as proposed in NENs of the digestive and pancreatic tract [19], as well as of the lung [13], but might also consider this novel and easily assessable morphological feature, the spread through air spaces.

Regarding the clinical outcome, the presence of STAS was significantly different in carcinoids that followed a significantly more aggressive course. This negative impact, at least in terms of OS, was independent from other negative prognostic factors including carcinoid histotype and was maintained also in the specific groups of TC and AC analyzed separately. In the clinical setting, this is particularly of interest for AC that are the most unpredictable category since they have an intermediate risk of recurrence and adverse outcome; in this group,

STAS may be used as an additional factor to predict a potentially aggressive behavior and to design the most appropriate therapeutic strategy, which apart from surgery is currently highly debated in this particular histological type [20, 21].

In fact, the current treatment landscape of AC beyond surgery remains largely uncertain and the lack of randomized studies, due to the rarity of the disease, does not allow establishing a solid treatment evidence. Current systemic approaches are quite individual and usually initiated at the time of the relapse or recurrence. The availability of prognostic markers indicating a more aggressive behavior may pave the way to an earlier systemic treatment initiation.

In conclusion, this is the first study that indicates a strong and independent prognostic value of the spread through air spaces (STAS), recently recognized in NSCLC, in lung carcinoids, with special reference to the atypical histological type.

Contributions Study conception and design: MP. Data collection: SA, JM, FM, GG, PC, GVS, MV, MP. Analysis and interpretation of data: MP, MV, SA, JM, MV. Drafting of manuscript: MP, MV, SA, JM. Critical revision: SA, JM, FM, GG, PC, GVS, MV, MP. All the authors gave final approval for publication. The author MV takes full responsibility for the work as a whole, including the study design, access to data, and the decision to submit and publish the manuscript.

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Compliance with ethical standards

The study was approved by the local ethical committee (Department of Oncology at San Luigi Hospital, number 17975/2015).

Conflict of interest The authors declare that they have no conflict of interest.

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