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Prognostic value of S-100 immunostaining in tumour cells of non-small cell lung cancer

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Abstract
S-100 protein expression is present in various malignant tissues, yet its prognostic relevance is debatable. The aim was to assess in non-small cell lung cancer (NSCLC) patients' prognostic value of S-100 protein considered alone or in relation with other variables. Tumour samples taken from 86 NSCLC patients during resection were assayed for S-100 protein expression with the use of polyclonal DAKO ZO311 antibody. S-100 expression was found in 32 cases (37%). Positive staining was not correlated with clinical characteristics including age, sex, pathology type of tumour, stage and cigarette smoking. There was a tendency for simultaneous expression of S-100 and *P53* protein ($p = 0.06$). A median survival rate for the entire group was 2.3 years (95% CI, 0.9–3.6 years). The median and 5-year survival of patients with positive staining for S-100 protein was 1.5 years and 25%, respectively, compared with 3.0 years and 35%, respectively, in the S-100 negative group ($p = 0.17$). In the final model of a multivariate analysis, S-100 protein expression in tumour cells was associated with significantly decreased survival ($p = 0.005$). S-100 protein expression in tumour cells seems to be an independent predictor of poor prognosis in NSCLC patients.

Keywords: *Non-small cell lung cancer, prognosis, p53 protein, S-100 protein.*

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Introduction

A clinical and pathological staging based on TNM classification is still the strongest predictor of survival in non-small cell lung cancer (NSCLC) patients. There is, however, a need for better characterization of factors influencing survival in specific subgroups and in individual patients. Recent studies addressing the prognostic value of biological markers in NSCLC have usually focused on molecular mechanisms regulating neoangiogenesis, proliferation and apoptosis, but only a few have addressed local immune response.

Infiltration of cells, including lymphocytes, dendritic cells (Langerhans cells, LhC) or fibroblasts within and around the tumour are morphologic features of host immune

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response against tumour cells (Ioachim et al. 1976, Nakamura et al. 1988, Johnson et al. 2000). The S-100 protein family is a group of small (10–12 kDa), highly homologous, acidic calcium-binding proteins (Zimmer et al. 1995, Schafer and Heizmann 1996). Most of the genes coding for S-100 proteins are clustered on human chromosome 1q21 (Schafer et al. 1995), and consist of three exons. They are involved in cytoskeletal membrane interaction, cell cycle progression, and differentiation and induction of apoptosis (Fano et al. 1995, Kligman and Hilt 1988). S-100 positive cells probably play an important role as antigen-processing cells in the induction of many types of T-lymphocyte-mediated response (Nakano et al. 1996). It is generally accepted that infiltration of LhC reflects a local cell-mediated immune response.

Although S-100 proteins were originally found in glial cells of the central nervous system and were proposed as a marker for neurogenic tumours (Camby et al. 1999), the S-100 protein-positive infiltration was shown in patients with various malignancies including squamous cell oesophageal cancer, breast and colorectal adenocarcinomas, and endometrial, nasopharyngeal, thyroid and cervical cancer (Furukawa et al. 1985, Schroder et al. 1988, Ambe et al. 1989, Matsushima et al. 1994, Coppola et al. 1998, Ikeguchi et al. 1998). Only a few studies have addressed S-100 expression in lung cancer (Zeid and Muller 1993, Coppola et al. 1998). In contrast to the favourable prognostic influence of increased S-100 expression in LhC, the presence of this protein in tumour cells seems to carry a negative prognostic impact (Kimura et al. 2000).

The present study was designed to assess the prognostic relevance of S-100 protein expression in primary tumour cells in NSCLC patients who underwent pulmonary resection.

Since multifunctional p53 protein is involved in almost the same vital functions in the cell as S-100 protein, we used the results of the former study that analysed p53 protein expression in this material (Jassem et al. 2000) and evaluated the co-expression of both p53 and S-100 proteins.

Material and methods

Patients

The study group included 86 consecutive patients (mean age 59 years, range 39–72 years) with microscopically confirmed NSCLC (Table I). All patients underwent pulmonary resection with curative intent at the Department of Thoracic Surgery, Medical University of Gdansk, Poland, in 1994. After surgery, one patient was assigned as stage IV disease, and was excluded from survival analysis. Pretreatment staging included chest X-ray, fibreoptic bronchoscopy, chest-computed tomography, and ultrasonography or computed tomography of the abdomen. The diagnosis was based on the current WHO classification and confirmed by two independent pathologists. In principle, postoperative irradiation or chemotherapy was not administered. However, a few patients received radiotherapy according to the individual decision of the treating physician. Paraffin-embedded tumour samples obtained from resected specimens were used for immunohistochemical analysis.

Table I. S-100 protein expression in relation to patient clinical characteristics.

Variable	Total	S-100 positive	*p*
Sex:			
Female	20	10 (50%)	0.14
Male	66	22 (33%)	
Histology:			
Squamous cell	48	20 (42%)	
Adenocarcinoma	29	9 (31%)	0.74
Large cell	7	2 (29%)	
Mixed	2	1 (50%)	
T-stage:			
1	8	3 (38%)	
2	62	24 (39%)	0.86
3	15	5 (33%)	
4	1	–	
N-stage:			
0	52	24 (46%)	
1	12	3 (25%)	0.19
2	21	5 (24%)	
3	1	–	
P53 expression:			
Positive	40 (47)	11 (28%)	0.065
Negative	46 (53)	21 (46%)	

Immunohistochemical staining

Tissue samples were fixed in 4% formalin and embedded in paraffin. Four-micrometre sections of the samples were deparaffinized and dehydrated consecutively in 95, 80 and 70% alcohol for 5 min. Standard haematoxylin-and-eosin assessment (to confirm the presence of tumour cells in the sample) was followed by incubation with polyclonal antibodies against S-100 proteins (1:400, DAKO Z0311) for 30 min. The presence of the antigen–antibody complexes was confirmed by biotinylated secondary antibodies and streptavidin-peroxidase complex (LSAB system, DAKO KO690). Diaminobenzidine (DAB) was used as the chromogen. All samples were stained with positive and negative controls.

Expression of S-100 protein was assessed as negative (−) when there was no positive staining, low (+) when fewer than 10% of the cells were stained, moderate (++) when 10–50% of the cells were positive, and high (+++) when more than 50% were positive (Figure 1).

For statistical analysis, low, moderate and high S-100 protein expressions were grouped together as 'positive'.

Statistical analysis

The computerized database included age, sex, smoking habits, tumour histology and grade, stage of disease, date of surgery, adjuvant treatment, date of last follow-up visit or death (verified in December 2004), S-100 protein and p53 protein expression.

Statistical analysis was performed with the use of statistical package *SPSS 9.0*. Chi-square and Fisher's exact tests were used for assessing the relation between S-100 and

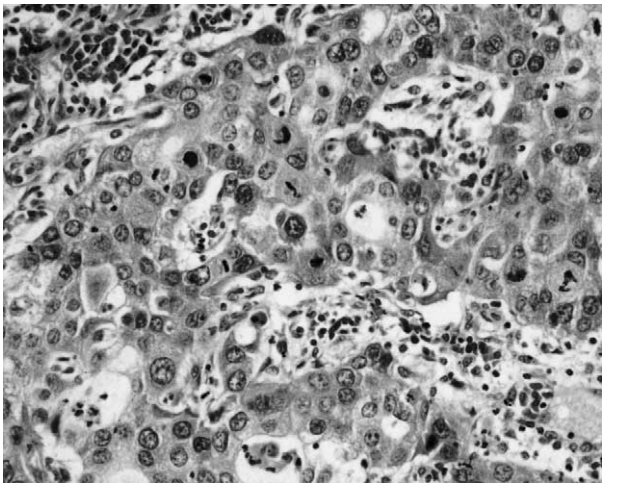

Figure 1. Non-small cell lung cancer with an adenocarcinoma-like pattern: moderate (++) patchy-type expression of S-100 protein in the cytoplasm and nuclei of most cells (×200).

p53 expression, and clinical variables. Survival time was calculated from the date of surgery to the date of death for any reason, or the date of the last follow-up. Survival curves were computed according to the Kaplan–Meier method and compared with the log-rank test. Multivariate analysis was based on the stepwise backward Cox proportional hazard model. For hypothesis testing, a two-sided $p < 0.05$ was considered as being statistically significant.

Results

Immunohistochemical findings

Positive immunostaining for S-100 in tumour cells was found in 32 of 86 samples (37%). Six tumours (7%) showed low, 21 (24%) moderate and five (6%) high-grade expression of S-100 protein.

There was no correlation between S-100 immunostaining and clinical characteristics (Table I). There was, however, a tendency to simultaneous over-expression of p53 and S-100 proteins ($p = 0.065$, Fisher's exact test).

Prognosis

By the time of analysis, 73 patients (85%) had deceased. The median survival rate in the entire group of patients was 2.3 years (95% CI: 0.9–3.6 years). The 3- and 5-year survival probabilities were 47 and 31%, respectively. The median survival of patients with positive staining for S-100 protein was 1.5 years, and the 5-year survival rate was 25% compared with 3.0 years and 35%, respectively, in the S-100-negative group (Figure 2). There was no statistical difference between S-100 staining and survival in univariate analysis (log-rank; $p = 0.17$). However, in the final model of multivariate analysis (including age, sex, histology, T and N status, smoking status and p53 expression), staining for S-100 protein and stage of disease (both T and N categories) were the only two factors significantly influencing survival ($p = 0.005$, and < 0.01,

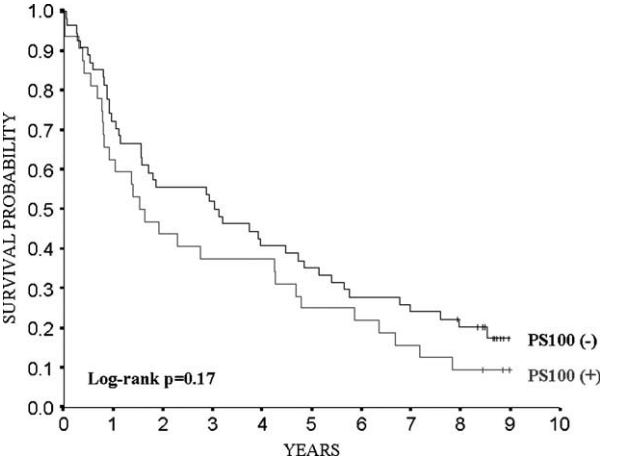

Figure 2. Survival according to S-100 status.

respectively; Table II). The addition of p53 protein expression data did not add to the predictive power of the multivariate model, as evaluated by the likelihood ratio statistic.

Discussion

Only a few studies have addressed S-100 protein expression in NSCLC. In the largest published series (68 patients), S-100 expression was found in 62% of squamous cell carcinomas, 55% of adenocarcinomas and 38% of large cell carcinomas (Nakajima et al. 1985). In the present study, the respective figures were somewhat lower: 41, 31 and 26%, respectively. The explanation for the expression of S-100 protein in lung cancer cells is unclear. S-100 contributes to immune response and is present in LhC (Furukawa et al. 1985, Nakajima et al. 1985). However, some of the S-100 protein family members also control a variety of cellular processes, such as cell-cycle progression, cell growth, proliferation and apoptosis. Their Ca-binding properties contribute to the modulation of many enzymes' activity. Interestingly, differences in some S-100 protein concentrations may stimulate adverse effects in the cell (Schafer and Heizmann 1996). Furthermore, some S-100 family members can form homo- or heterodimers, which are expressed in a cell-specific way. In addition, the mechanisms of S-100 protein expression are not uniform. The coding sequences of the S-100 family members are highly homologous but the sequence of introns and promoters

Table II. Multivariate analysis, final Cox model.

Variable	Hazard ratio	95% CI	*p*
S-100 staining	2.10	1.25–3.53	0.005
T1	1.0	–	–
T2	2.51	0.88–7.13	0.084
T3	5.51	1.72–17.66	0.004
N0	1.0	–	–
N1	1.37	0.66–2.85	0.401
N2	3.81	2.03–7.18	<0.009

does not show much resemblance. Some S-100 genes are expressed only in certain cell types and this is based on the recognition of *cis*-acting elements in a gene promoter by cell specific transcription factors (Lesniak et al. 2000). On the other hand, in some S-100 genes, particular promoter fragments may act as cell specific repressors (Castets et al. 1997). Cell-specific expression may also be regulated by epigenetic processes, such as DNA methylation or chromatin organization (Wicki et al. 1997). Thus, it may be concluded that the over-expression of S-100 protein usually reflects pathological changes (Camby et al. 1999), as was demonstrated in carcinomas other than lung cancer (Zoltowska et al. 1996, Mueller et al. 1999). It is also possible that particular members of the family may contribute to different steps of carcinogenesis. S-100A9 and S100A9 proteins were found in early prostate cancers, and their serum levels discriminated between cancer and benign hyperplasia of the prostate (Hermani et al. 2005). In contrary, S100A4 was found to stimulate metastatic spread of tumour cells in a genetically modified mouse model (Ambartsumian et al. 2005). Further, in the clinical study, higher expression of this protein was related to greater aggressiveness of colorectal carcinoma (Cho et al. 2005).

No correlation was found in this series between S-100 expression and clinico-pathological characteristics. In one study, S100A4 expression positively correlated with NSCLC stage (Kimura et al. 2000). In contrast, laryngeal squamous cell carcinoma immunostaining for S100A2 was lower in less advanced and high-grade tumours (Lauriola et al. 2000). Interestingly, in this series a trend was found to a simultaneous occurrence of positive immunostaining for S-100 and p53 proteins. The explanation for this finding is difficult. Polyclonal antibody Dako Z 311, which was used in this study, reacts with both the A and B groups of S-100 proteins (genes located on chromosome 1 are numbered as S-100A1, S-100A2, etc., whereas genes located on chromosome 3 or elsewhere are numbered S-100B). However, the question as to which individual protein is responsible for increased immunostaining remains unanswered. It is also likely that the expression of protein reflects an abnormal S-100 gene transcription or results from an increased 'need' for protein in response to tumour growth.

The prognostic significance of S-100 positive immunostaining in NSCLC remains unclear. The expression of S-100 proteins in tumour stroma is considered a favourable prognostic factor (Zeid and Muller 1993, Honig et al. 2005). For example, Zeid and Muller demonstrated that a high number of S-100 positive dendritic cells in tumour tissue were associated with prolonged survival. However, the statistical analysis in their study merely included a simple comparison of the overall survival in the group with the highest and lowest number of DC. Moreover, both groups were highly heterogeneous and included NSCLC and SCLC patients. Nevertheless, the authors concluded that increased number of S-100 positive dendritic cells reflects an active immunological state, which may herald longer survival.

In the present study, univariate analysis did not demonstrate significant influence of S-100-positive immunostaining on survival in NSCLC patients, but a multivariate Cox model showed a significant adverse impact of this feature. The explanation of this discrepancy may be related to the higher proportion of advanced stage tumours in the S-100 negative group (metastatic involvement of mediastinal nodes was found in 30% in the S-100 negative group compared with 16% in the S-100 positive group; Table I). When corrected for stage in the multivariate model, S-100 protein expression became significant, with an adjusted hazard ratio of 2.1. Clearly, further studies including

larger number of patients would be required to confirm this finding. Another issue warranting investigation is the relevance of the expression level. In the present study, we used a semi-quantitative method of expression assessment, but the analysis of particular subgroups was not performed due to relatively small patient's sample.

Recent studies brought a new insight into the role of S-100 proteins at early stages of lung carcinogenesis (Feng et al. 2001, Heighway et al. 2002). It was demonstrated that S-100A2 expression was suppressed possibly by methylation of its promoter (Feng et al. 2001). 5-Azacytidine has been recently tested as a probable agent inhibiting abnormal methylation of some S-100 proteins (Lesniak et al. 2000, Feng et al. 2001).

In summary, the present study demonstrated a negative impact of S-100 expression in NCSLC cells on survival. The clinical usefulness of this marker considered alone or in combination with other assays warrants further investigation.

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