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# Serum p53 antibodies in small cell lung cancer: the lack of prognostic relevance

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**Abstract**

Prognostic relevance of serum p53 antibodies was assessed in 96 patients with microscopically proven small cell lung cancer (SCLC). The study group included 67 males and 29 females; mean age 58 years; range 35–86 years; 60 with limited disease (LD), and 36 with extensive disease (ED). The control group consisted of 41 patients with non-malignant diseases. The presence of p53 antibodies was assayed by the immunoenzymatic method (P53 ELISA kit, PharmaCell, France). Antibodies were present in 26 SCLC cases (27%); 15 (25%) in LD and 11 (31%) in ED. Antibodies were also found in one out of 41 control subjects (2%). There was no correlation between the level of antibodies and clinical characteristics of SCLC patients including age, gender and extent of disease. The median follow-up for the entire group was 30 months (range: 11–39 months). By the time of analysis, 78 patients (82%) had deceased. Median survival in SCLC patients with and without antibodies was 42 and 39 weeks, respectively (log rank, $P = 0.81$). These results indicate the lack of clinical relevance of serum p53 antibodies in SCLC. 

*Keywords:* Serum autoantibodies; p53 antibodies; Small cell lung cancer; Prognostic value

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## 1. Introduction

*P53* gene mutation is a common event in tumorigenesis and occurs in approximately 80–90% of small cell lung cancers (SCLC) [1]. This abnormality almost always leads to synthesis of a structurally and functionally changed protein which is more stable than normal wild-type protein. The consequence of these processes is a stimulation of immunological response expressed by the production of specific antibodies [2,3].

In contrast to the large body of clinical data on *P53* gene mutation and p53 protein expression [4], the role of p53 antibodies in this tumor remains highly undefined. Frequency of serum antibodies against p53 protein in lung cancer ranged in particular series from 7 to 70% [1,5–7]. The prognostic value of pre-therapy serum level of p53 antibodies was demonstrated in some tumors [8–10], including non-small cell lung cancer (NSCLC) [11–13]. The value of these tests in predicting survival in SCLC has been addressed only occasionally [14]. The present study was designed to evaluate prospectively the prognostic value of p53 antibodies in SCLC in relation to other clinical characteristics: age, gender and extent of the tumor.

## 2. Material and methods

The study group included 96 consecutive patients (67 males and 29 females, mean age 58 years, range from 35 to 86 years) with cytologically or histopathologically diagnosed SCLC (Table 1). Most of the patients were primarily diagnosed at two departments of thoracic surgery in Gdańsk and Szczecin. Disease extent was determined according to the criteria proposed by the Veterans Administration Lung Group with modification by the International Association for the Study of Lung Cancer. Staging procedures included physical examination, chest X-ray, abdominal ultrasonography or computed tomography, and bone marrow biopsy. Brain CT, bone scintigraphy and other tests were optional in asymptomatic patients and mandatory in those with symptoms suggesting dissemination. Blood tests included morphology, renal function assays, electrolytes, transaminases, alkaline phosphatase and lactate dehydrogenase. There were 60 patients with limited disease (LD) and 36 with extensive disease (ED). All patients were administered one of the three standard chemotherapy regimens: cyclophosphamide, epidoxorubicin and etoposide; cyclophosphamide, methotrexate, vincristine and CCNU; or cisplatin and etoposide. Patients with LD were administered chest irradiation after the completion of chemotherapy. Prophylactic cranial irradiation was not routinely performed within the analyzed period. Fifteen patients underwent surgical intervention prior to systemic treatment (five, resection of the tumor, seven, lobectomy and three, pneumonectomy). Most of those cases included patients with small peripheral lesions in whom final diagnosis of SCLC was established

Table 1
The presence of p53 antibodies in relation to clinical characteristics of patients

| Characteristics | Total number of patients | P53(+) number of patients (%) | *P*-value ($\chi^2$-test) |
|---|---|---|---|
| *Age* | | | |
| $<50$ years | 20 | 7 (35) | |
| $\geq 50$ years | 76 | 19 (25) | 0.37 |
| *Gender* | | | |
| Male | 67 | 17 (25) | |
| Female | 29 | 9 (31) | 0.57 |
| *Disease extent* | | | |
| LD | 60 | 15 (25) | |
| ED | 36 | 11 (31) | 0.55 |

only after the pathological examination of a surgical specimen.

The control group included 41 patients with non-malignant disorders (20 males and 21 females, mean age 58 years, range from 47 to 75 years), treated at the Outpatient Clinic for Internal Diseases at the Medical University in Gdańsk.

Blood samples (5 ml) were collected from each patient prior to treatment along with the routine tests, and stored after centrifugation at −24°C. The level of p53 antibodies was determined with the use of ELISA for autoreactive human p53 antibodies kit (PharmaCell, France). Patients' sera, 1:100 diluted, were added for 1 h at room temperature to microplate wells coated with either the human, recombinant p53 protein or control extract. After washing, an antihuman IgG peroxidase conjugate was added and incubated for 1 h at 37°C. The bound enzymatic activity was determined by adding a 3,3′5,5′-tetramethylbenizidine substrate. The reaction was stopped after 10 min. Absorption was measured at 504 nm using a MR 5000 Elisa reader (Dynatech Lab.). Results were expressed as a ratio between the value of the wells with p53 and the corresponding wells without p53. According to the recommendation of PharmaCell, France, a sample was considered positive for anti-p53 antibodies if its absorbance value was $\geq 1.6$ with index $\geq 1.1$ (the index was calculated as the ratio between the specific signal of sample and the specific signal of weak positive control provided with the kit). The sensitivity and specificity of this assay have been described earlier [15,16].

Samples were analyzed blindly in duplicates at the Medical University of Gdańsk and at the Institute Curie, Paris, with the final concordance of 100%. The level of p53 antibodies in analyzed sera was expressed as a relative p53 autoimmune index.

All clinical parameters were recorded in the data base and analyzed with the use of two statistical packages: Statistica 5.0 and S-PLUS 2000. Non-parametrical Mann–Whitney *U*-test was used for comparisons of antibodies' levels between particular groups, and $\chi^2$-test for evaluating the relation between p53 antibodies and other clinical characteristics. Survival was assessed by the Kaplan–Meier method and compared with log-rank test. Multivariate analysis was performed according to Cox regression with stepwise backward procedure. Hazard ratio and its 95% confidence interval for each variable were estimated by Cox proportional hazard model. A *P*-value lower than 0.05 was considered statistically significant.

## 3. Results

Twenty-six (27%) out of 96 SCLC patients were positive for serum p53 antibodies; 15 (25%) with LD and 11 (31%) with ED ($P = 0.55$). The proportion of positive results in SCLC was markedly higher than in the control group (one out of 41 subjects, 2%; $P < 0.001$). Accordingly, the mean index value in SCLC group (2.30; range: −0.81 to 23.07) was significantly higher than in controls (−0.34; range: −2.80 to 3.29, $P < 0.0001$). No significant difference in the mean index level was found between LD and ED groups (2.16 and 2.54, respectively, $P = 0.86$). The presence of antibodies was not related to age, gender and stage of disease (Table 1).

Survival data were available for 95 patients. Median follow-up of censored observations was 30 months (range: from 13 to 39 months). By the time of this analysis, 78 patients (82%) had died. Median survival for the entire group was 40 weeks. One- and 2-year survival probability was 44% (95% CI: 34–54%) and 18% (95% CI: 10–27%), respectively. At the cut-off point recommended by the provider of the assay, no influence of p53 antibodies presence on survival was noted (median survival in patients with and without antibodies of 42 and 38 weeks, respectively; $P = 0.81$). One-year survival rates in both groups were 46 and 43% and 2-year survival rates, 22 and 17%, respectively (Fig. 1). Also when the test result was evaluated as a continuous variable, no relation with survival was observed (relative risk of 0.97; 95% confidence interval: 0.93–1.02). Thus, there was no prognostic relevance of the test when different cut-off values were considered. Furthermore, there were no significant differences in survival between patients with and without p53 antibodies when the groups were analyzed accord-

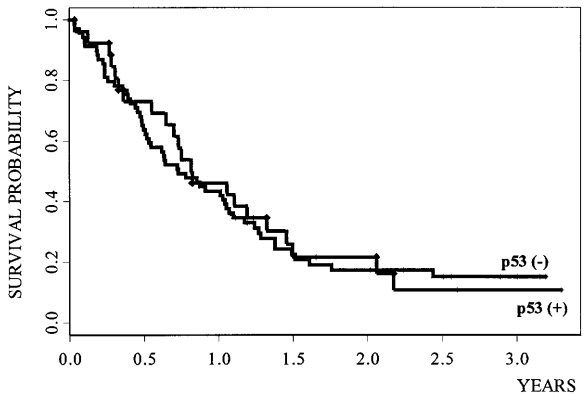


Fig. 1. Survival in relation to the status of serum p53 antibodies.

ing to the extent of disease (log-rank; $P = 0.64$ and 0.22 for LD and ED, respectively). The following variables were analyzed in univariate and multivariate analysis: age, gender, disease extent, and level of serum p53 antibodies. The only significant prognostic factor for survival both in univariate and multivariate Cox analysis was disease extent ($P = 0.048$; Table 2). When surgery (performed in selected patients with limited disease) was incorporated into the Cox model, this variable was shown to be the strongest predictor of survival ($P = 0.006$).

## 4. Discussion

Our study was designed to evaluate whether humoral response against mutant p53 protein is of clinical relevance in SCLC. Most of the patients in this series were diagnosed at surgical departments, therefore the proportion of LD patients was higher than in the general population of SCLC in Poland. This skewed proportion, however, did not obscure the final outcome as the level of antibodies was not related to the extent of disease.

The idea of potential prognostic usefulness of serum p53 antibodies seems to be of particular relevance in poor-prognosis malignancies such as SCLC. Serum p53 antibodies were shown to have a relatively high specificity in human tumours [6,16]. In one study the only two control individuals with present p53 antibodies developed malignancy during 1 year of follow-up [17]. In the present series, only one out of 41 healthy controls had a positive result of the test. This person, a 51-year-old male, heavy smoker, who suffered from ischemic heart disease, underwent careful investigation which did not demonstrate any malignancy. The patient was also free from any autoimmune and collagen disease. No tumor developed within the next 12 months of follow-up.

In contrast to their high specificity, the sensitivity of p53 antibodies is in most malignancies relatively low. In the present study, abnormal levels of serum p53 antibodies were found in 27% of SCLC patients; more frequently than in the largest study addressing this tumor (27 of 170 patients; 16%) [14]. However, in other smaller series the incidence of positive results in SCLC ranged from 8 to 71% [5–7]. Comparison between the outcomes in particular studies is difficult because of different methods used for measuring the level of p53 antibodies. In our study the mean antibody index value in SCLC patients was 2.3. The value reported by Segawa et al. [6] was higher (9.8), but their series included only seven SCLC patients. Similarly to other series [5,7] we did not

Table 2
Univariate and multivariate analysis of survival, $n = 95$

| Variable | Univariate analysis | | | Multivariate analysis (final model) | | |
|---|---|---|---|---|---|---|
| | Relative risk | 95% CI | *P*-value | Relative risk | 95% CI | *P*-value |
| Age | 1.02 | 0.99–1.04 | 0.22 | – | | |
| Gender | 0.79 | 0.48–1.29 | 0.34 | – | | |
| Disease extent | 1.59 | 1.01–2.51 | 0.048 | 1.59 | 1.01–2.51 | 0.048 |
| Serum anti-p53 antibody | 0.94 | 0.57–1.54 | 0.81 | – | | |

Table 3
Prognostic value of serum p53 antibodies in various malignancies

| Author (reference) | Tumour[a] | Total number of patients (% of patients with p53 antibodies) | Prognostic impact (multivariate analysis) | Remarks |
|---|---|---|---|---|
| Rosenfeld et al. [14] | SCLC | 170 (16) | No | |
| Present study | SCLC | 96 (27) | No | Survival analysis included 95 patients |
| Winter et al. [21] | SCLC | 36 (58) | No | In univariate analysis tendency to better survival in a group with p53 antibodies ($P = 0.059$) |
| Lai et al. [5] | 14 SCLC, 111 NSCLC | 125 (8) | No | Significant negative impact in the subgroup of patients with advanced disease |
| Laudanski et al. [12] | NSCLC surgically resected | 84 (23) | Negative ($P = 0.001$) | |
| Mitsudomi et al. [13] | NSCLC surgically resected | 188 (20) | No | Survival analysis included 171 patients; in multivariate analysis, significantly shorter survival ($P = 0.02$) in the subgroup with both antibodies against N-terminal region of p53 protein and p53 antibodies |
| Komiya et al. [11] | NSCLC | 140[b] | No | In multivariate analysis, significantly shorter survival ($P = 0.033$) in the subgroup with squamous cell carcinoma and in those with presence of p53 antibodies combined with the lack of p53 protein expression |
| Bergqvist et al. [19] | NSCLC treated with radiotherapy | 67 (27) | Not reported | Positive impact of p53 antibodies univariate analysis ($P = 0.025$) |
| Kressner et al. [8] | Colorectal cancer | 184 (32) | No for entire group; Negative for radically resected | Negative impact in univariate analysis ($P = 0.03$); trend towards negative impact in multivariate analysis ($P = 0.07$) |
| Lenner et al. [9] | Breast cancer | 165[b] | Negative | Survival analysis included 99 cases |
| Gadducci et al. [22] | Ovarian cancer | 86 (10) | No | |
| Maehara et al. [10] | Gastric cancer | 120 (19) | No | Negative impact in univariate analysis ($P < 0.05$) |

[a] SCLC, small cell lung carcinoma; NSCLC, non-small cell lung carcinoma.
[b] The distribution of p53 antibodies index levels was presented.

find a correlation between the presence of antibodies and clinical variables including age, gender and stage of disease. One study including both SCLC and NSCLC demonstrated a higher incidence of p53 antibodies in patients with malignant pleural effusion [5]. The presence of pleural effusion was not recorded in our series, therefore we did not include this variable in the analysis.

The use of serological methods, as opposed to molecular and immunohistochemical methods does not require a tissue sample and may be performed in a routine diagnostic procedure. Moreover, antibodies may be used to monitor patients during treatment [17,18]. The principal aim of the present investigations was to evaluate the prognostic value of p53 antibodies. Our results indicate that this marker is not related to survival in SCLC patients; the finding is consistent with the aforementioned study of Rosenfeld et al. [14]. The prognostic value of p53 antibodies was demonstrated in some malignancies but this association usually concerned only selected subsets of patients (Table 3). Thus, the prognostic relevance of p53 antibodies in human malignancies is probably limited. Most likely the main reason for these negative results is the relatively rare occurrence of this marker. In SCLC the frequency of serum p53 antibodies seems to be significantly lower than the rate of mutated p53 protein in tumor cells. Despite its low sensitivity, this assay was recently demonstrated as a useful tool in chemotherapy monitoring in patients with advanced NSCLC [18]. In that study, a decrease of p53 antibodies during therapy was observed in 12 out of 16 patients. Moreover, patients with decreasing p53 antibodies tended to have a better response to treatment and prolonged survival. Another study suggested the value of p53 antibodies in predicting response to radiotherapy [19]. A possible diagnostic role of p53 antibodies includes detection of early lung cancer, for example in the high risk group of COPD patients smoking cigarettes [17,20].

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