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Low level anti-Hu reactivity: a risk marker for small cell lung cancer?

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Abstract

Background—Previous experimental and laboratory studies have implicated antibodies against Hu proteins (anti-Hu) as a potential marker for small cell lung cancer (SCLC); there are no estimates of the association between anti-Hu and SCLC using a population-based design.

Methods—We used stored plasma specimens to evaluate anti-Hu reactivity in relationship to small cell lung cancer in a population-based case-control study. Using Western Blot analysis, we measured anti-Hu reactivity against recombinant Hu family member, HuD, in plasma samples from forty-one (41) SCLC cases and seventy-nine (79) controls individually matched for age, race, sex, and smoking status (never, past, current). We analyzed the association between anti-Hu reactivity and SCLC using conditional logistic regression.

Results—Anti-Hu reactivity was associated with SCLC, both before and after adjustment for amount of smoking. We observed a smoking-adjusted odds ratio of 3.2 (95 % confidence interval from 0.98 to 13.4) comparing subjects above 1800 units (the lower limit of the second tertile of the distribution among antibody positive controls) to subjects with lower reactivity. We also found suggestive evidence in follow-up of our cases that anti-Hu above 1800 units was related to longer-term survival from SCLC. The present research is the first report of anti-Hu reactivity and SCLC in a population-based study.

Conclusions—Given the suggestive evidence in this study, prospective analyses to examine whether anti-Hu reactivity might predict risk of developing SCLC, or whether anti-Hu reactivity could serve as an early marker for SCLC, may be warranted.

Keywords

carcinoma; small cell; Hu paraneoplastic encephalomyelitis antigens; HuD antigen; autoantibodies; case-control studies; survival

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Introduction

Establishment of association with the disease in question is an important first step in biomarker identification. Biomarkers should ideally reflect and/or predict the disease with high specificity and sensitivity [1]. Results from small clinical samples can provide important first clues to potential biomarker-disease associations that can later be examined in larger study designs.

Lung cancer is the leading cause of cancer death in the United States and Western Europe. Small cell lung cancer (SCLC), showing properties of primitive neuroendocrine cells [2], accounts for up to 13% of all newly diagnosed lung cancers [3] and is strongly associated with cigarette smoking [4–8]. Initially, SCLC patients respond well to chemotherapy, however, relapses are inevitable and are usually resistant to cytotoxic treatment; only 10% of all SCLC patients have significant long-term survival [9].

Paraneoplastic encephalomyelitis/sensory neuronopathy (PEM/SN) is one of a number of rare paraneoplastic autoimmune diseases associated with SCLC. PEM/SN is characterized by dementia, sensory loss, and other neurological disabilities [10]. SCLC patients with PEM/SN have high titers of antibodies that react against neuronal nuclear proteins of 35–40 kDa known as Hu proteins [11;12]. Hu proteins are a family of four RNA-binding proteins, three of which--HuB/Hel-N1, HuC, and HuD-- are normally restricted to the nervous system, although HuB/Hel-N1 has also been detected in the testes and ovaries [13]. Hu proteins are homologous to the embryonic lethal abnormal visual (elav) protein in Drosophila and play a role in neuron-specific RNA processing and neural development [12;14–16]. In SCLC, Hu antigens are abnormally expressed in the tumor, characterizing them as onconeural antigens. Generation of anti-Hu autoantibodies is thought to be part of an immune response which cross-reacts with the healthy nervous system, resulting in PEM/SN [17]. The neurological disorder, rather than the cancer, is usually the cause of death in SCLC patients with PEM/SN [18].

All SCLC tumors, whether from patients with or without PEM/SN, express neuronal Hu proteins [12;19;20]. Dalmau et al. and Graus et al. found that ~16% of SCLC patients without paraneoplastic neurological autoimmune syndromes have detectable titers of anti-Hu antibody in their serum, albeit at much lower levels than PEM/SN patients [10;21]; additional studies using similar techniques conducted by Verschuuren et al and Monstad et al found anti-Hu reactivity in 17% and 25.5% of SCLC cases, respectively [22;23]. No studies have yet evaluated anti-Hu antibodies among healthy subjects from population-based studies, and overall population prevalence is unknown.

Although anti-Hu antibodies are also found in a fraction of neuroblastoma patients, they are rarely present in other cancers. Thus, the presence of anti-Hu antibodies in patient serum may serve as a marker for SCLC, and as a model for antibody-based early cancer detection, and may function as a prognostic indicator. Other paraneoplastic diseases, such as Lambert-Eaton myasthenic syndrome (LEMS) [24;25], limbic encephalomyelitis (LE) [26;27], opsoclonus myoclonus syndrome [28], and cancer-associated retinopathy [29;30] are also associated with SCLC, indicating that SCLC patients may express additional cancer-specific antibodies against neuronal proteins [31]. In theory, if a large enough panel of SCLC-associated antigens could be identified, these antigens could carry potential value for early detection of this disease. Because SCLC is so rapidly metastatic, it has been argued that early detection (and ensuing intervention) of SCLC is not feasible. However, because the process by which SCLC develops is unknown, the possibility that such antibodies might be detectable in SCLC at a stage prior to rapid disease progression cannot be excluded.

To determine whether anti-Hu antibodies are associated with increased risk of developing SCLC, we studied the anti-Hu response in a population-based case-control study that included healthy smoking and non-smoking controls and small cell lung cancer cases. To this end, we

analyzed anti-Hu antibody levels in 41 SCLC patient plasma samples and 79 matched population controls. We additionally studied the association between anti-Hu antibodies and survival time among the cases.

Materials and Methods

Plasma Samples and Study Population

Plasma specimens were previously collected from a population-based case-control study of African Americans and Caucasians from Los Angeles County, California, that was conducted during the early to mid-1990s [32]. Participation in the study included provision of a blood sample and completion of an in-person interview on risk factors for lung cancer, including smoking history. Plasma had been stored at −80°C since the original blood collection. Among the 342 lung cancer cases that participated in the original study, 46 were diagnosed with SCLC. Stored plasma was available for 41 SCLC cases.

Because we wanted to examine whether smoking was associated with anti-Hu reactivity among the controls, we selected two control groups (smokers and never smokers). Controls were individually matched to SCLC cases on age (within 5-year age group), race, and gender. The first set of controls consisted of current smokers or former smokers, matched to the smoking status of the case. The second control group consisted of never smokers. If multiple smoking and non-smoking controls matched to a SCLC case, one control of each was chosen at random. We analyzed plasma from 120 subjects including 79 healthy controls (40 smokers and 39 non-smokers) and 41 SCLC cases.

Written informed consent was obtained from each study participant. The University of Southern California institutional review board approved the use of human subjects in accord with an assurance filed with and approved by the US Department of Health and Human Services.

Purification of HuD recombinant protein

Anti-Hu sera cross-react with all three neuronal Hu antigens, which are highly conserved [20]. While all SCLCs express neuronal Hu antigens, HuD was reported to be most commonly expressed [20]. Thus we determined anti-HuD reactivity in our samples using recombinant HuD protein, prepared as described [33], with the following modifications. The sonication buffer consisted of 20mM HEPES (pH 7.4), 150mM NaCl, and 0.5% Triton X-100. Proteins were eluted from Ni^{2+} beads using sonication buffer containing 10% glycerol and 50–250mM imidazole. The protein concentration was determined by the Bradford assay (Bio-Rad Laboratories, Hercules, CA) and verified on Coomassie blue-stained gels by running dilutions of the purified protein along a similar molecular weight standard of known concentration.

Western Blotting

Protein (1ug in 20ul total volume) was resolved on 10% sodium dodecyl sulfate (SDS) gels and transferred to nitrocellulose filters as described in Towbin et al. with the following modifications [34]. Transfer was performed for 45 minutes at 100 V while chilling buffer with a cold pack. The filters were blocked with 3% bovine serum albumin (BSA) in Tris-buffered saline, Tween-20 (TBST: 10 mM Tris/HCL pH 8.0 150mM NaCl, 0.05% Tween-20) overnight at room temperature. The following day, blots were incubated with 1:1000 dilution of plasma in 3% BSA/TBST for 2 hours at room temperature followed by three washes for 10 minutes with 0.3% BSA/TBST at room temperature. Secondary antibody, anti-human horse-radish peroxidase (Sigma Product No. A-0170), was diluted 1:2000 in TBS and added for 1 hour. After washing five times for 6 minutes in TBS, the blot was submerged in SuperSignal® West Substrate Working Solution, prepared according to the manufacturer's instructions (Pierce,

Product No. 34080). The blots were imaged using Bio-Rad Fluor-S™ MultiImager, and images were taken every 20 seconds during a 5 minute exposure time

Determination of Anti-Hu Antibody Reactivity

Previously, it was shown that use of recombinant HuD in a Western blot analysis is the most sensitive and specific method for the detection of anti-Hu activity [10;35]. Older analyses in which neuronal lysates were used run the risk of showing reactivity to unknown proteins of similar molecular weight. Even when using affinity purified HuD from bacterial lysates, occasionally background bands of the wrong size are observed. Caution must therefore be used when determining anti-Hu reactivity. In our analysis, only plasma samples showing a band of the correct size (~40 kDa for the tagged recombinant HuD) were scored as reactive against Hu proteins.

To quantitate the anti-HuD reactivity on the Western blots, we used the Quantity One 1-D Analysis Software Version 4.4.1 (Bio-Rad, Product No. 170–9600). Using this software, we drew a box around the band of interest at 35- to 40-kDa molecular mass in the Western blot image. This same box was copied onto a nearby area with no signal to provide a quantitation for background signal. The background was subtracted from the band-box and this value was used as the adjusted reactivity reading. This was done for each individual Western blot image. Values were set at zero for individuals with an adjusted reading value of zero or less than zero. Western blots were analyzed and quantitated blindly at the same exposure time of five minutes. Representative examples are shown in Figure 1.

Follow-Up of Study Population

We performed follow-up of all participants to determine mortality and incidence of new cancers. Using the cut-off date of December, 31, 2003, we checked each study record against information from the SEER Cancer Registry, USC Cancer Surveillance Program of Los Angeles County, for mortality, cause of death, date of death, cancer diagnosis, and date of diagnosis after original study participation.

Statistical Analysis

The SAS statistical software package was used to perform tabular and quantitative analyses [36]. We compared frequency of detection of anti-Hu reactivity according to case-control status. Because the sample sizes were small, we used Fisher's Exact Test in conjunction with McNemar's statistic to evaluate departure from expected [37]. We used SAS to fit conditional exact logistic regression models of the association between anti-Hu reactivity and SCLC, adjusting for amount smoked.

To evaluate the association between anti-Hu reactivity and risk of small cell lung cancer, we treated no reactivity as the baseline referent category, or linear zero, and constructed approximate tertiles based on the distribution of antibody positive among the controls (>0 – 1800; 1800 – 5400; >5400). The median value of anti-Hu reactivity for each category was used to model tests for trend. Because the logistic regression estimates were not different for the upper two tertiles, we collapsed the anti-Hu reactivity variable into two categories: 0 – 1800 and > 1800 [38].

Case-control differences in levels of covariates were examined with pair-wise ANOVA t-tests for continuous variables, and McNemar's chi-square statistic for categorical variables [37]. To assess case-control differences in anti-Hu reactivity, we constructed a variable to indicate values of anti-Hu reactivity above zero as 'detectable', and values of anti-Hu reactivity equal to zero as 'undetectable.' Tests of statistical significance were two sided, and a p-value of less than 0.05 was considered significant.

The potential role smoking might play in relationship to the association between anti-Hu reactivity and SCLC had never before been evaluated. Here our goal was to carefully account for residual confounding from smoking. Due to our small sample size, we chose matching as a method to partially balance our control distribution for smoking history. We matched one set of controls to each case for smoking status (current smoking; former smoking). Because we wanted to compare whether anti-Hu reactivity differed according to smoking, we added a second set of controls who were non-smokers. Models were further adjusted for smoking amount by including indicator terms for total pack years of smoking (< 40 pack years; 40+ pack years).

We conducted survival analysis using the product limit method of estimation for the small cell cases and anti-Hu reactivity in relationship to time to death or censorship since original lung cancer diagnosis. The linear shape of the negative-logarithm plot of survivor function for all small cell cases suggested that the exponential distribution assumptions were met. We constructed survival distribution plots and used likelihood ratio tests to assess difference between survival curves for anti-Hu reactivity. We constructed a Cox proportional hazards regression model adjusting for sex and amount smoked. Additional terms for age and ethnicity added to the proportional hazards model did not improve precision or result in changes to effect estimates, and they were therefore not included in the model.

Results

Characteristics of SCLC cases and controls are summarized in Table 1. The matching factors of race, age, and sex were correspondingly similar between cases and controls. As expected, pack-years of smoking was slightly greater among cases than smoking controls, although this difference was not statistically significant ($p = 0.13$). Results from the Western blots for anti-Hu reactivity are shown by case-control status in Figure 2.

Table 2 contains the distribution of detectable anti-Hu reactivity for cases and both control groups. Among the total study population, significant differences were observed between cases and control groups ($p = 0.01$) for detectable anti-Hu reactivity, ranging from 66% among the cases, to 46% among the smoking controls, and 39% among the non-smoking controls. The same trend was observed in the racial, gender, and age subgroups, but was most pronounced in younger subjects (<60 years of age), with cases having 73% detection of anti-Hu; smoking controls with 29% detection; and non-smoking controls with 29% detection ($p = 0.02$). We also observed significant differences in mean anti-Hu reactivity for cases and controls among Caucasians ($p=0.02$) and among male subjects ($p=0.02$).

In order to evaluate whether smoking was related to anti-Hu reactivity, we compared the frequency of detectable anti-Hu reactivity for the smoking and non-smoking controls (supplementary data[1]). Anti-Hu reactivity did not vary by smoking for the total sample ($p = 0.41$) nor did anti-Hu reactivity vary according to racial, or gender subgroups.

Higher anti-Hu reactivity was associated with SCLC, both before and after adjustment for smoking amount (Table 3). The risk of SCLC tended to increase across categories of anti-Hu up to 1800–5400 units, but dropped at the uppermost category, > 5400 units (p for trend = 0.06). We observed a smoking adjusted odds ratio of 3.2 (95 % confidence interval from 0.98 to 13.4) comparing subjects with anti-Hu reactivity > 1800 to subjects with lower reactivity.

We conducted follow-up on small cell lung cancer cases, in order to determine whether anti-Hu reactivity was related to prognosis. Figure 3 contains product-limit estimates of survival

[1]Supplementary Table 1 will be published only in the journal's "on-line" version.

from small cell lung cancer according to anti-Hu reactivity. Cases with an anti-Hu reactivity level > 1800 units survived longer (median survival time = 15.6 months) than cases below 1800 anti-Hu reactivity level (median survival time = 11.4 months) (p = 0.08). The follow-up period was too short with too few controls to assess whether anti-Hu reactivity might be associated with future development of small cell and other cancers.

We further examined the association between survival time and anti-Hu reactivity by constructing a Cox proportional-hazards regression model and estimating hazard-rate ratios for the probability of surviving. The hazard rate ratio in relation to anti-Hu reactivity levels above 1800 units was 0.46 (95% confidence interval = 0.23 to 0.93) compared to below 1800 units, adjusting for sex and smoking amount.

Discussion

This is the first examination of anti-Hu reactivity in relation to lung cancer risk in a population-based study. To sensitively determine levels of anti-Hu reactivity in SCLC cases and control subjects, we used recombinant HuD protein in a Western blot analysis, using Biorad Quantity One 1-D Analysis Software to quantitate 5-min exposures in a blinded fashing. Only reactivity against the correct molecular weight band was scored as positive. This assay allows the sensitive and specific unbiased detection of even low titers of anti-Hu antibodies while minimizing false positives that may be obtained with other methods [35]. For example, as was done in the Dalmau study, the use of crude neuronal extracts may lead to scoring of sera with reactivity against proteins other than Hu [21;39], while in ELISA the size of the reacting protein is not visible. The use of imaging software likely increased our sensitivity compared to other studies, which often score samples using a more subjective scals (e.g. “mild, moderate, intense”) (see below).

In our study, we did not establish a ‘reactivity’ cut point based on reactivity levels in control subjects. Instead our goal was to measure the direct level of anti-Hu reactivity in all subjects and evaluate associations between level of anti-Hu reactivity and SCLC. In contrast. Dalmau and colleagues considered reactivity of normal human sera (equivalent to 6U/ml) as background; sera from SCLC patients with and without PEM/SN were considered positive only if their reactivity was greater than twice the background value found in normal sera. Seven of 44 (16%) SCLC patients without PEM/SN had detectable levels of anti-Hu antibody (average 76 U/ml) [21]. Other studies that used recombinant HuD antigen on Western blots created categories of mild, moderate, or intense to describe anti-Hu reactivity [10;22]. These studies identified positive reactivity in 16% of their total SCLC population [10], and 17% of SCLC cases [22]. Guidelines for detection of anti-Hu antibodies established by the International Society of Neuro-Immunology noted that specimens yielding no reactivity on immunohistochemistry may nonetheless be detected by Western blot analysis using highly affinity purified recombinant proteins, suggesting that these methods may be more sensitive [35]. When using a highly sensitive in vitro transcription-translation (ITT) assay and immunoprecipitation, Monstad et al, observed anti-Hu reactivity in 25.5% of SCLC patients. When they submitted the same specimens to a dot blot analysis using recombinant HuD and 1:2000 dilution, they found results (18.5%) comparable to previous studies [23].

Methods for classifying reactivity and detection methods thus appear to impact prevalence of anti-Hu reactivity. To make compare our analysis to other studies, we could consider the highest anti-Hu reactivity value in the non-smokers as our baseline cutoff value (~7500). In cutting our reactivity at 7500 and considering values above 7500 as positive, we found that 15% of SCLC patients had detectable antibody levels. This finding is similar to previous results [10; 21;22], but, in our opinion, negates the important finding that anti-Hu reactivity is detectable at values lower than 7500 among smoking and non-smoking controls.

We found a moderate association between anti-Hu reactivity and SCLC. The odds ratio for moderate to high positive anti-Hu reactivity (>1800 units), adjusted for total pack years of smoking, was marginally statistically significant (odds ratio = 3.2 (95% confidence interval = 0.98 to 13.4)).

Examining the frequency of detectable anti-Hu reactivity, we observed an unadjusted association between anti-Hu reactivity and case-control status ($p = 0.01$). We adjusted for total pack years of smoking using analysis of covariance (ANCOVA), and the association between continuous anti-Hu reactivity and case-control status was significant ($p = 0.05$). We compared anti-Hu reactivity among the controls according to smoking status. We found no overall difference and no differences when we stratified by gender and race. In the retrospective studies by Dalmau and Verschuuren, there was a predominance of women who were anti-Hu positive [21;22]. Graus et al observed no difference in mean age and sex in SCLC patients with and without PEM/SN [10]. When comparing patients positive or negative for anti-Hu antibodies, Monstad et al reported no significant differences between sex or age [23]. These studies did not however examine anti-Hu reactivity according to race.

We observed that anti-Hu reactivity was protective for survival from small cell lung cancer in our study, however results can only be considered suggestive due to small numbers. Our results are in agreement with the previously observed longer survival time of anti-Hu positive SCLC patients [22], the more limited disease observed in anti-Hu positive SCLC cases [21], and reports about small cell lung cancer cases with paraneoplastic sensory neuronopathy in complete remission [40;41]. The presence of anti-Hu antibodies may indicate an immune system response to small cell lung cancer that could function to prolong survival.

Our study differs from previously conducted research on anti-Hu antibodies and SCLC in that we used healthy population-based controls matched on age, race, sex, and smoking status. Large scale studies are needed to determine whether anti-Hu reactivity profiles can serve as an early detection marker for SCLC. Interestingly,, one of the smoking controls with a detectable level of anti-Hu antibody, healthy at time of study participation, died of SCLC several years after the study was concluded. This observation supports a possibility that moderate level anti-Hu reactivity might be an indicator for future SCLC. While this is an anecdotal occurrence, appearance of a small cell lung cancer case in a control group of this size would not be expected given the underlying background of SCLC incidence in Los Angeles County that occurred during the study period [3;42].

We found that smoking was not associated with anti-Hu reactivity among our healthy controls. Smoking greatly increases the risk of small cell lung cancer. Development of anti-Hu reactivity, on the other hand, may signal the presence of a small cell lung tumor, but it is not necessarily related to smoking.

Small cell lung cancer is one of the most rapidly fatal cancers [3;9]. Identification of high-risk smokers that may develop SCLC could help to improve survival. Smokers who are determined to be at risk for SCLC could be closely monitored and could serve as candidates for chemoprevention once effective agents become available. In addition, they should strongly be encouraged to quit smoking. Although only suggestive, given our small sample size, reactivity against Hu proteins and other auto-antigens could indicate the first signs of a growing small cell lung tumor, potentially in time to prolong survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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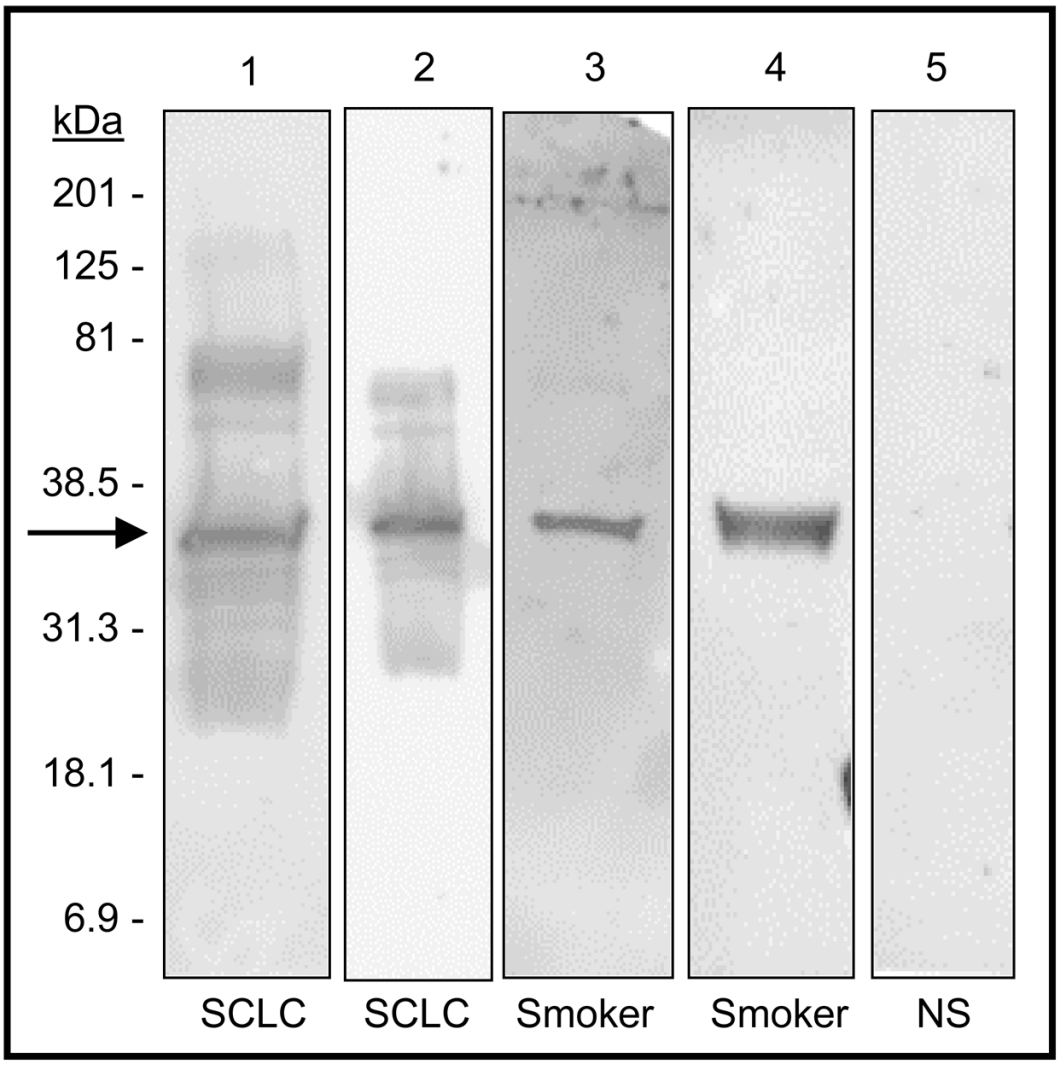

Figure 1.
Representative examples of anti-Hu immunoreactivity assays of plasma from SCLC cases and controls. Blots of recombinant human HuD were probed with 1:1000 dilutions of plasma. Lanes 1 and 2: plasma from two SCLC cases. Lanes 3 and 4: plasma from two smoking controls. Lane 5: plasma from a non-smoking control that showed no detectable reactivity. For quantitation, 5 minute exposures were used. For the purposes of this figure only to illustrate that exposure time did not affect reactivity, different exposure times are shown here: 1 min, 40s, 40s, 9 min, and 20 min respectively from left to right.

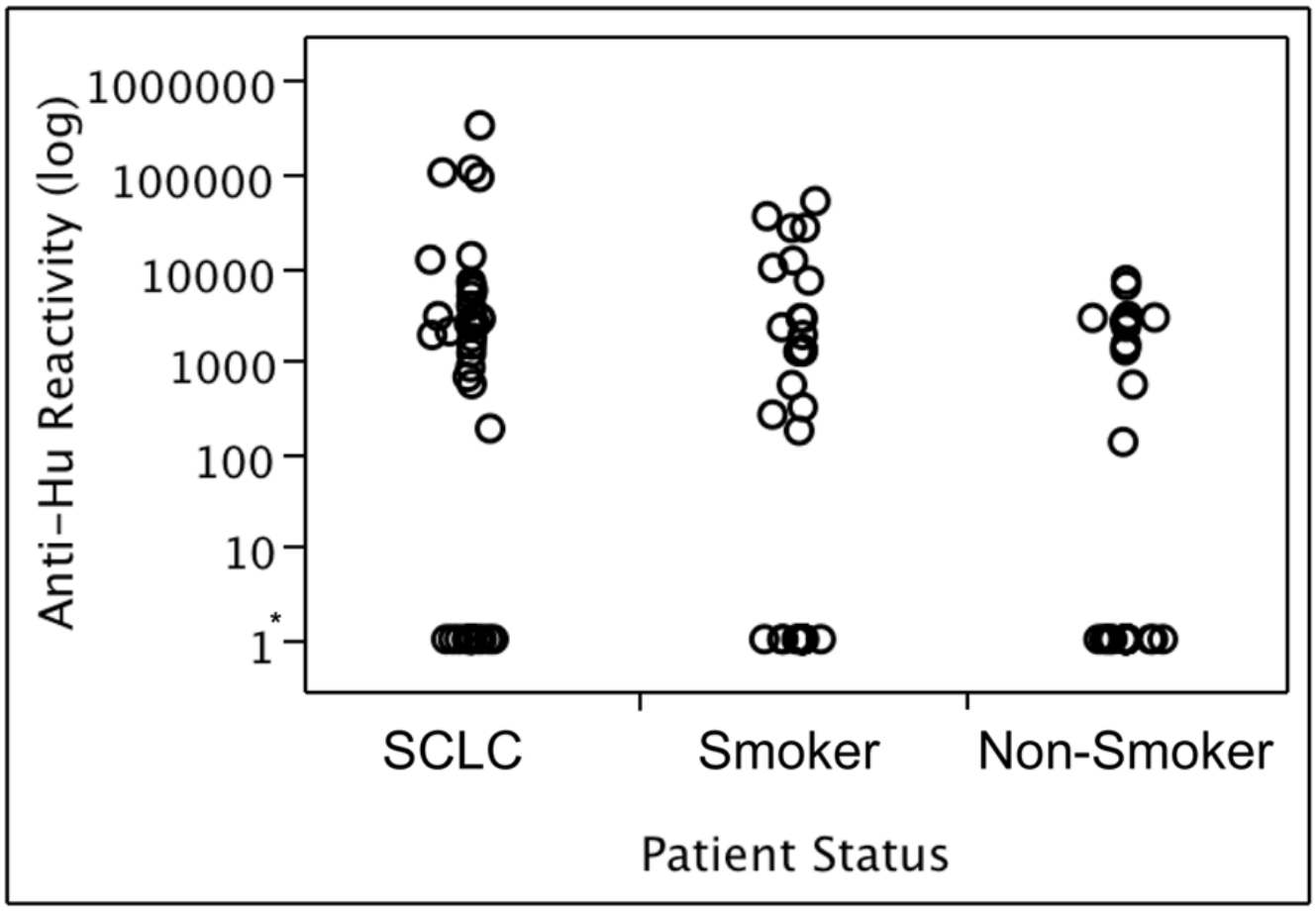

Figure 2.
Anti-Hu reactivity levels in SCLC cases, smoking controls and non-smoking controls plotted on a logarithmic scale. To represent arithmetic zero, 1.0 was added to each adjusted reading value.

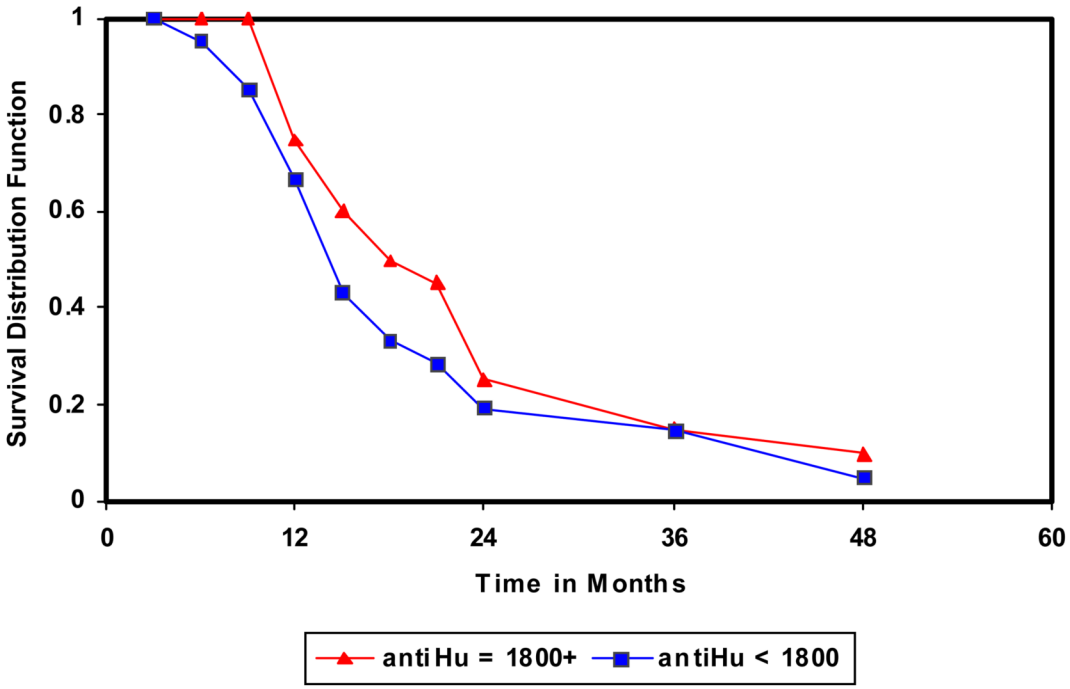

Figure 3.
Plot of time until death or censorship. Plots of survival functions for anti-Hu reactivity; above and below 1800 units.
X-axis indicates time in months. Y axis indicates survival distribution function.

Table 1

Distribution of Covariates According to Case-Control Status

Variable	Category	Cases		Smoking Controls		Non-Smoking Controls		Total Controls		
		N	%	N	%	N	%	N	%	p-value[1]
Race	Caucasian	27	65.85	25	65.79	25	65.79	53	67.95	
	African American	14	34.15	13	34.21	13	34.21	25	32.05	0.82
Gender	Male	21	51.22	19	50.00	18	47.37	42	53.85	
	Female	20	48.78	19	50.00	20	52.63	36	46.15	0.79
Smoking Status	Never	0	0.00	0	0.00	39	100.00	39	50.00	
	Former	8	19.51	7	17.95	0	0.00	7	8.97	
	Current	33	80.49	32	82.05	0	0.00	32	41.03	0.86[2]
Age (in years)	< 60	15	36.59	15	39.47	14	36.84	28	35.90	
	60 – 69	20	48.78	19	50.00	20	52.63	41	52.56	
	70 +	6	14.63	4	10.53	4	10.53	9	11.54	0.97
Age (in years)	Mean		62.46		62.31		61.68		62.31	
	S.D.		8.33		8.07		7.34		7.42	0.86
Pack years	Mean		47.17		37.66		0.00		18.83	
	S.D.		29.97		25.03		0.00		25.85	0.13[2]

[1] McNemar exact p-value for categorical data, and pair-wise ANOVA p-values for continuous data from cases and total matched controls (smoking and non-smoking)

[2] McNemar exact p-value for categorical smoking status and paired t-test for mean pack years from cases and matched smoking controls

Table 2

Frequency of Detectable Anti-Hu Reactivity According to Covariate and Case-Control Status

Variable	Category	Anti-Hu Reactivity	Cases		Smoking Controls		Non-Smoking Controls		
			N	%	N	%	N	%	p-value[1]
Total Population		Undetectable	14	34.2	21	53.9	24	61.5	
		Detectable	27	65.9	18	46.2	15	38.5	0.01
Race	**Caucasian**	Undetectable	9	33.3	14	53.9	16	59.3	
		Detectable	18	66.7	12	46.2	11	40.7	0.02
	African American	Undetectable	5	35.7	7	53.9	8	66.7	
		Detectable	9	64.3	6	46.2	4	33.3	0.30
Gender	**Male**	Undetectable	6	20.0	12	60.0	12	54.6	
		Detectable	15	80.0	8	40.0	10	45.5	0.02
	Female	Undetectable	8	40.0	9	47.4	12	70.6	
		Detectable	12	60.0	10	52.6	5	29.4	0.21
Smoking Status	**Former**	Undetectable	2	25.0	3	42.9			
		Detectable	6	75.0	4	57.1			0.66[2]
	Current	Undetectable	12	36.4	18	56.3			
		Detectable	21	63.6	14	43.7			0.14[2]
Age (in years)	**< 60**	Undetectable	4	26.7	10	71.4	10	71.4	
		Detectable	11	73.3	4	28.6	4	28.6	0.02
	60 – 69	Undetectable	8	40.0	9	42.9	13	65.0	
		Detectable	12	60.0	12	57.1	7	35.0	0.11
	70 +	Undetectable	2	33.3	2	50.0	1	20.0	
		Detectable	4	66.7	2	50.0	4	80.0	0.29

[1] McNemar exact p-value for categorical data from cases and total matched controls (smoking and non-smoking)

[2] McNemar exact p-value for categorical smoking status from cases and matched smoking controls

Table 3

Conditional [1] exact odds ratios describing the association between anti-Hu reactivity and small cell lung carcinoma

Variable	Category	Cases	Controls	OR [2]	OR [3]	95% Confidence Interval [3]	p-value[4]
Anti-Hu Reactivity	**0**	14	45	1.00	1.00	----------	
	> 0 – 1800	7	13	1.96	0.74	0.10 – 4.40	
	1800 – 5400	11	11	3.55	5.44	1.06 – 54.36	
	> 5400	9	9	3.94	1.77	0.39 – 9.39	0.06
Anti-Hu Reactivity	**0 – 1800**	21	58	1.00	1.00	----------	
	> 1800	20	20	3.10	3.17	0.98 – 13.38	

[1] Cases matched to controls for age (five-year intervals), race, sex, and smoking status

[2] Unadjusted

[3] Adjusted using indicator variables for pack-years

[4] Score Test Probability