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Autoimmunity to SOX2, clinical phenotype and survival in patients with small-cell lung cancer

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ABSTRACT

Objective: Autoantibodies to SOXB1 antigens are commonly found in patients with small-cell lung cancer (SCLC). It has not been established whether the presence of circulating SOX antibodies is associated with a specific paraneoplastic clinical phenotype, or if a tumour immune response to SOX antigens can affect prognosis in patients with SCLC in relation to other established prognostic factors.
Methods: Using recombinant SOX2 in an ELISA, we analysed sera in a prospective study from 212 unselected SCLC patients, which included 35 patients with neurological paraneoplastic disorders, or other well characterised onconeural antibodies.
Results: Overall, SOX2 antibodies were detected in 70 SCLC patients, with a sensitivity of 33% (95% CI 27–40%) and specificity of 97% (95% CI 94–99%) compared to controls matched for age, gender and smoking history. No single clinical phenotype was seen in relation to the presence of SOX2 antibodies in isolation. Multivariate analysis showed that the presence of SOX2 antibodies in SCLC patients without evidence of neurological paraneoplastic disorders or onconeural antibodies did not have a significant effect on survival when known prognostic factors were accounted for.
Conclusions: SOX2 antibodies are very specific markers for SCLC compared to matched non-tumour controls, but their presence does not seem to alter prognosis in this tumour type.

Lung cancer is the most common cause of death from malignancy in the UK [1]. The incidence of small-cell lung cancer (SCLC) in this population is approximately 20%. Despite the often dramatic response to chemotherapy and radiotherapy in patients with SCLC, most die from recurrent disease [2]. With rapid tumour doubling rate and early metastasis, over half of all patients with SCLC present with extensive disease [3]. As a result, 5-year survival statistics in SCLC are still very poor, at only 7% in England and Wales in 2000–2001, with median survival reported as being as low as 4 months at a regional UK centre [1,4].

Computed tomography screening programmes have indicated that earlier detection of lung tumours significantly improves long-term survival, although these studies were not randomised, with no control group [5]. A recent review of the role of surgery in SCLC has suggested that a combination of surgery and chemotherapy in early stage disease may see 5-year survival rates of up to 73% in patients with stage 1A cancers [6], highlighting the benefits early diagnosis of such cancers may yield.

Autoantibodies to tumour-associated antigens have been detected in up to 68% of patients with SCLC [7], sometimes reported to be present prior to the presence of symptomatic disease [8,9]. The presence of autoantibodies has also been linked to prognosis in lung cancer, although it is not clear whether the presence of a host's immune response to tumour antigens improves overall survival. In patients with SCLC, the presence of p53 autoantibodies has been associated both with poor survival [10] and no change in prognosis [11]. Likewise, the presence of anti-Hu antibodies has been associated with improved survival [12], although this was not confirmed in other studies [13,14].

In patients with autoimmune paraneoplastic neurological disorders (PNDs), the identification of distinct clinical syndromes such as Lambert-Eaton myasthenic syndrome (LEMS), usually evident before tumour detection, has been linked to improved survival compared with SCLC patients without PNDs [15]. However, it is not clear whether it is the lead time bias in detecting lung tumours early, or an effect on the tumour from circulating antibodies (such as voltage-gated calcium channel antibodies (VGCC) in LEMS) that improves long-term survival.

Recently, a number of studies have identified the presence of SOXB1 group antibodies in populations of patients with SCLC, with and without PNDs, including LEMS [16–19]. SOXB1 (SOX1, SOX2,

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SOX3) Sry-like high mobility group box proteins are transcriptional factors, involved in early neurogenesis, and expressed in neuronal precursor cells and adult human cerebellum [20]. These antigens have been shown to be highly immunogenic in SCLC [19]. Limited retrospectively collected data in two studies [16,18] have shown that the presence of SOX antibodies in SCLC patients is not significantly associated with improved survival, although the number of healthy control sera tested was small, and the proportion of male patients in these cohorts was higher than that expected from recent population studies on incidence of SCLC [3]. One of the striking findings from these studies was the high incidence of SOX antibodies found in SCLC patients with LEMS (64–67%) [16,17], although the numbers studied were too small to determine whether survival was different in these patients compared with SOX-antibody-negative LEMS patients [16].

In this study of SCLC patients, we aimed to establish whether the presence of SOX antibodies in a large, prospectively assessed cohort of SCLC patients was associated with a novel neurological paraneoplastic phenotype, and if the presence of SOX antibodies was associated with any improvement in tumour survival.

1. Methods

1.1. Small-cell lung cancer patients

Two hundred and twelve unselected patients with biopsy-proven SCLC, who consecutively consented to the study, were recruited at time of tumour diagnosis from hospitals within the Trent region of the UK (Nottingham Research Ethics Committee approval 04/Q2404/100). Patient recruitment began in May 2005. All patients underwent full neurological evaluation and examination, and serum samples were taken prior to chemotherapy, and stored at −70 °C for further analysis. Additional patients with characteristic PNDs were included in the study if further follow-up investigations revealed SCLC. Any patient initially included in the study who subsequently developed new neurological symptoms was seen again for review. Follow-up clinical data were obtained on all patients, and death dates were taken from medical records.

For antibody analysis, the control group consisted of two hundred and twelve healthy volunteers recruited in the UK who were matched to the SCLC patients according to age, gender and smoking history, and had no evidence of any current or prior cancer. Serum samples were collected from all control individuals and stored at −70 °C prior to analysis.

1.2. Paraneoplastic neurological syndromes

Patients were diagnosed with LEMS if they showed typical clinical features of the disease, with or without electrophysiological confirmation, in line with standard published diagnostic features [21,22]. Other PNDs, such as sensory neuronopathy, limbic encephalitis, or paraneoplastic cerebellar degeneration, were diagnosed according to previously published clinical and immunological diagnostic criteria [23].

1.3. Antibody analysis

Autoantibodies to VGCCs in serum from patients with SCLC or controls were measured by immunoprecipitation of VGCCs extracted from human cerebellum and labelled with either 125I-ω-CmTx MVIIC (P-/Q-type VGCC) or 125I-ω-CgTx GVIA (N-type VGCC) as previously described [24]. Antibody titres greater than 3SD above normal and disease controls (50 pM) were considered positive.

Antibodies to onconeuroantigens HuD, Yo, Ri, CV2 (CRMP5), amphiphysin and Ma2 were detected using an commercial immunoblot kit. (RAVO Diagnostika GmbH, Freiburg, Germany).

Autoantibodies to SOX2 were detected by the use of a semi-automated ELISA; briefly recombinant SOX2 antigen, and a control (tag only) were used to coat microtitre plates. These were probed with serum samples and autoantibodies were detected with anti-human IgG [7]. Positivity was defined as a (non-specific binding/background corrected) signal 3.5SD above the mean of the normal control group that was reproducible on at least two separate occasions, with inter-assay reproducibility achieving 98–100%.

1.4. Statistical analysis

Group statistics were compared using Fisher's exact test for frequency distribution, and Mann–Whitney *U* test for mean numerical values. To evaluate the clinical characteristics of patients purely with SOX2 antibodies, those with additional onconeural or VGCC antibodies, with or without clinical features of PNDs or LEMS were excluded. Differences in survival according to SOX2 status were evaluated using Stata v10 (StataCorp, Stata Statistical software v10.1, College Station, Texas USA, Stata Corporation) by means of a Cox proportional hazards model incorporating known clinical predictors of survival (age, gender, Karnofsky score and extent of disease) as covariates. Missing values of LDH and sodium were multiply imputed using the Stata add-on *ice* [25,26]. Further model fitting was performed based on minimising the Akaike Information Criterion (AIC) in order to identify a parsimonious mode that predicted survival [27].

2. Results

2.1. Patient characteristics

Over two-thirds of all eligible patients with SCLC diagnosed in the study period consented for inclusion in this study. Of the 212 patients with a new diagnosis of SCLC, the mean age was 67 years (range 33–87), 111 (52%) were males, and 125 (59%) had extensive disease. Twenty-five patients overall (12%) had an associated autoimmune condition, most commonly thyroid disease and rheumatoid arthritis. Sixteen patients (8%) presented with central nervous system (CNS) metastases, with a similar number developing CNS disease spread on follow-up.

Lambert-Eaton myasthenic syndrome was present in nine patients (4%), all of whom had positive titres of VGCC antibodies. A further 11 patients had detectable VGCC antibodies, but no clinical evidence of LEMS, even during follow-up, on re-examination (although one patient developed limbic encephalitis, with no other detectable onconeural antibodies). Classical PNDs were detected in 4 patients (sensory neuronopathy in two, limbic encephalitis in one, and a motor neuron disease-like illness in one), and well-defined onconeural antibodies were detected in a further 11 patients with no clinical evidence of PNDs, either initially, or on follow-up. All 15 of these patients had detectable anti-Hu antibodies, except one with Anti-Ri and another with anti-amphiphysin antibodies, neither of which had a PND.

2.2. Clinical features and immunoreactivity to SOX2 antibodies

The specificity and sensitivity of the SOX2 assay for the detection of SCLC in all serum samples (212 SCLC and 212 normal) were 97% (95% CI 94–99%) and 33% (95% CI 27–40%) respectively.

SOX2 antibodies were detected in 52 of 177 SCLC patients (29.3%, 95% CI 23–37%) with no detectable classical PND or onconeural antibodies. Only six of the age-, gender-, and smoking history-matched normal control serum samples were positive for SOX2 antibodies (3%, 95% CI 1–6%). Univariate analyses of known prognostic factors and SOX2 reactivity in 177 SCLC patients with no PND or onconeural antibodies are shown in Table 1. Of these

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Table 1
Univariate analyses.

Prognostic factor	Value	Number of patients	Median survival	Hazard ratio	LCL	UCL
Serum LDH	Raised (greater than 450 U/L)	80	9.25	2.18	1.26	3.75
	Normal	34	18.5			
Gender	Male	93	9	1.24	0.87	1.76
	Female	84	10.25			
Age group	>68 years (median)	82	8.5	1.62	1.14	2.30
	Less than 68 years	95	10.75			
Performance status	Poor	78	8	2.12	1.49	3.02
	Good (Karnofsky score 80, 90, 100)	99	14			
Extent of disease	Extensive	107	8.5	1.96	1.35	2.85
	Limited disease	70	14			
SOX2 antibody	Negative	125	9	0.82	0.56	1.22
	Positive	52	13			

Abbreviations: LDH, lactate dehydrogenase; LCL, lower 95% confidence limits; UCL, upper 95% confidence limits.

Table 2A
Cox regression analysis (analysis with only complete data ($n = 114$)).

Prognostic factor	Hazard ratio	Standard error	z	*P*-value (two-sided)	LCL	UCL
Ageband	1.10	0.28	0.39	0.70	0.67	1.82
Gender	1.23	0.30	0.85	0.39	0.76	2.00
Extent of disease	1.68	0.44	1.98	0.05	1.01	2.79
Performance status	0.55	0.14	−2.36	0.02	0.33	0.90
Raised LDH	1.79	0.52	1.98	0.05	1.01	3.17
SOX2	0.85	0.23	−0.60	0.55	0.50	1.45

177 cases, the median age of SOX2-positive patients was lower at 63.5 years compared with SOX2-negative patients (69 years, univariate analysis, $P = 0.022$), and antibody-positive patients were more likely to be female ($P = 0.04$). The proportion of females in the SOX2-positive group (57.7%) was greater than in the 177 SCLC patient study group as a whole (47.5%), but not significantly so ($P = 0.21$). Associated autoimmune diseases were found in similar numbers of SOX2-positive (13%) and SOX2-negative patients (11%). The proportion of patients presenting with limited disease at initial SCLC diagnosis was similar in both SOX2-positive (37%) and SOX2-negative (41%) patients.

Initial and follow-up clinical neurological examination revealed no single pure phenotype associated with SOX2 positivity. Proximal limb weakness in isolation was detected in a greater proportion of SOX2-positive (8/52, 15%) than SOX2-negative patients (6/125, 5%, $P = 0.028$). None of these 14 patients had any other detectable antibodies, and one patient tested had no electrophysiological evidence of LEMS, or other neuromuscular disorders. SOX2 antibodies were not associated with an ataxic syndrome. Autonomic symptoms were described in similar proportions between the SOX2-positive (10%) and SOX2-negative (14%) groups.

SOX2 antibodies were detected in most (7/9, 78%) LEMS patients, while only 36% (4/11) were SOX2-positive if VGCC antibodies were detected in the absence of clinical or electrophysiological evidence of LEMS. Only 47% (7/15) of patients with other onconeural antibodies (Hu, Ri, Amphiphysin) also had SOX2 antibodies. Patients with SOX2 antibodies and LEMS had a median survival time of 18.25 months (range 1.75–48).

Univariate survival statistics calculated on the 177 SCLC patients who had no other detectable onconeural or VGCC antibodies showed that patients with positive SOX2 antibodies had longer median survival (13 months, 95% CI 9–17) compared to SOX2-negative patients (9 months, 95% CI 8.25–10), although this did not reach significance (Log rank test $P = 0.189$).

The results of the Cox proportional hazards model of prognostic factors (Table 2A) show that the effects of SOX2 positivity on survival were still not significant when known prognostic factors were taken into account (177 SOX2 patients: hazard ratio 0.85, $P = 0.55$, 95% CI 0.50–1.45). As LDH values were missing in some patients, the elevated LDH value was multiply imputed and this analysis (Table 2B) reduced the magnitude of the SOX-2 positivity prognostic value even further. The only variables showing an association with outcome in both the multivariable analyses were performance status and extent of disease. Fig. 1A illustrates the fitted SOX2-positive and SOX2-negative survival, assuming proportional hazards and adjusting for all the other prognostic variables, while Fig. 1B shows the result of adjusting only for extent of disease and performance status.

Table 2B
Cox regression analysis following multiple imputation for raised LDH ($n = 177$).

Prognostic factor	Hazard ratio	Standard error	*z*	*P*-value (two-sided)	LCL	UCL
Ageband	1.37	0.27	1.62	0.105	0.94	2.01
Gender	1.30	0.24	1.44	0.151	0.91	1.88
Extent of disease	1.74	0.34	2.79	0.005	1.18	2.56
Performance status	0.57	0.11	−2.97	0.003	0.39	0.83
Raised LDH	1.44	0.35	1.52	0.131	0.90	2.32
SOX2	0.97	0.20	−0.15	0.877	0.64	1.46

Fitting a reduced model with just extent of disease, performance status and SOX2 give a hazard ratio for SOX2-positive cases of 0.84 (0.57–1.25).
Abbreviations: LDH, lactate dehydrogenase; LCL, lower 95% confidence limits; UCL, upper 95% confidence limits.

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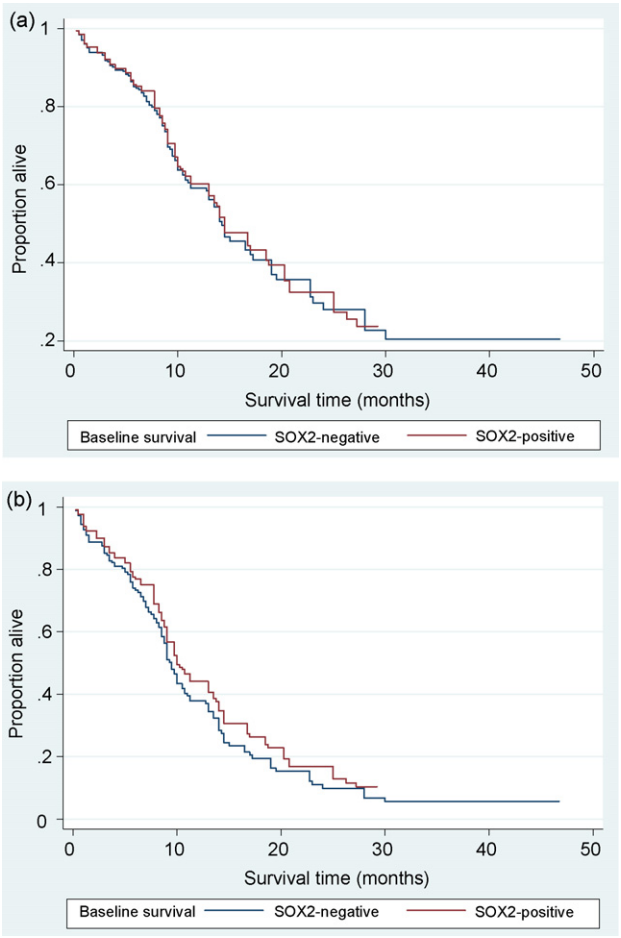

Fig. 1. (A) Fitted survival of SOX2-positive and SOX2-negative patients adjusted for ageband (younger or older than median age of 68 years), gender, LDH, extent of disease and performance status. (B) Fitted survival of SOX2-positive and SOX2-negative patients adjusted for the significant prognostic factors of extent of disease and performance status.

3. Discussion

In this large, prospective study of unselected SCLC patients, we have shown that immunoreactivity to SOX2 was present in 33% of patients. The presence of SOX2 antibodies was associated with a non-significant increase in survival compared with SOX2-negative patients, and SOX2 antibody-positive patients were more likely to be female, and younger. There was no single, distinct neurological paraneoplastic phenotype that was found in SOX2-positive patients, although SCLC patients who had additional antibodies associated with PNDs, in particular LEMS, were much more likely to harbour SOX2 antibodies (up to 78%). There was no association between SOX2 immunoreactivity and increased prevalence of other autoimmune conditions.

To date, there have been four studies of the presence of SOX antibodies in patients with SCLC [16–19], only two of which included data from control patients [16,18], but our series represents the first prospective study of SOX immunoreactivity in relation to clinical phenotype and survival in a large cohort of patients with a new diagnosis of SCLC. As a result, our unselected study patients represent an accurate sample of a typical SCLC population, in terms of age at onset, proportion of patients with limited disease at initial diagnosis, and gender distribution, even within the same geographical region from previous published data [4]. Previous studies of SOX immunoreactivity in SCLC have included larger proportions of males [16,18] than would now be expected in a contemporary SCLC cohort [3]. An over-representation of men in studies of SOX antibodies in SCLC patients may lower the expected sensitivity of SOX immunoreactivity for SCLC compared to controls.

By using a large number of controls, matched for age, gender, and smoking history, we were able to obtain a statistically robust measurement of SOX2 sensitivity, compared with previously published data. It is recognised that a range of autoantibodies may be detectable in healthy subjects, with evidence suggesting an increase in autoantibody levels with increasing age [28,29]. It is not certain whether the increased incidence of autoantibodies is related to immunoregulatory changes of ageing, or increasing incidence of cancer in older people. We also matched SCLC and control patients for gender as it is known that women display a different autoimmune disease profile relative to men [30]. Overestimation of SOX2 sensitivity in patients with SCLC in our study was avoided by considering these factors when establishing a matched control patient group.

Prospective analysis of SOX2 antibodies has meant we have been able to establish, through initial neurological evaluation and on subsequent follow-up clinical review on SOX2 antibody-positive patients, that there is no distinct paraneoplastic neurological phenotype associated with SOX2 antibodies. Intriguingly, some patients seemed to have a forme fruste of LEMS, with proximal limb weakness, but neurophysiological evaluation on follow-up failed to detect a defect in neuromuscular transmission. It would be expected that an intracellular antigen such as SOX2 would not have a direct pathogenic role, certainly in terms of clinical effects on the nervous system. SOX immunoreactivity typically stains the nuclei of Bergmann glia of the cerebellum [17], but no SOX2-positive patients in this current study, or in previous reports, have developed ataxia or other features of cerebellar dysfunction.

Analysis of immunoreactivity to SOX2 in the 177 SCLC patients who had no other PNDs or commonly associated antibodies allowed us to determine whether development of antibodies to tumour-associated antigens improved survival, in a patient group where lack of pre-SCLC diagnosis clinical features such as PNDs, conferring lead time bias, would not be a factor. We observed that although survival was improved in SOX2-positive patients, this did not reach significance. This was not attributable to the greater proportion of women and younger patients in this seropositive group because the hazard ratio estimated in the Cox regression analysis (which takes the mutual correlations of these variables into account) was little different from the univariate hazard ratio, while following multiple imputation for the missing LDH values the hazard ratio for SOX2 positivity moved even closer to unity. The coefficient of variation for the SOX2 hazard ratio estimate was 20%, suggesting that the estimate was reasonably precise and that a clinically significant decrease in hazard could be ruled out. Improved survival did not correlate with high ELISA SOX2 titres (data not shown). There was no bias towards presentation with limited disease in SOX2-positive patients.

We have demonstrated the usefulness of SOX2 immunoreactivity with high specificity for SCLC compared with a control population matched for age, gender, and smoking history, in a prospective study, allowing us to make accurate assumptions about clinical phenotype and effects of SOX2 seropositivity on survival. It would seem that the presence of a lead time bias from the occurrence of a clinical PND (such as LEMS) before the diagnosis of SCLC is the most important factor in conferring an improvement in survival. Subclinical immunoreactivity to tumour antigens such as SOX2 (with no pure, clinical PND phenotype) is not a favourable prognostic factor in SCLC.

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Conflict of interest

Caroline Chapman and John Robertson are consultants to Oncimmune Ltd., a University of Nottingham spinout company.

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