Chapter 11

P/Q-type calcium channel antibodies, Lambert-Eaton myasthenic syndrome and survival in small cell lung cancer

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Abstract
To assess the survival impact of the presence of P/Q-type calcium channel antibodies in patients with small cell lung carcinoma (SCLC), we examined the frequency of the antibodies and Lambert-Eaton myasthenic syndrome (LEMS) in 148 consecutive patients with SCLC, and in 30 patients with paraneoplastic cerebellar degeneration and SCLC, and studied their relation with survival. In both series, only patients with LEMS had a remarkably long survival, whereas presence of the antibodies without LEMS did not result in a better prognosis.
Introduction
Small cell lung carcinoma (SCLC) cells express P/Q-type (Ca,V2.1) voltage-gated calcium channels (VGCCs), against which the majority of patients with the Lambert-Eaton myasthenic syndrome (LEMS) produce antibodies that are functionally active and responsible for the typical muscle weakness. LEMS usually antedates the detection of SCLC and occurs in a small proportion of SCLC patients. LEMS was associated with improved survival in patients with SCLC, indicating that in LEMS the immune response to the tumour might inhibit its growth, thereby increasing the survival. A small subgroup of SCLC-patients had P/Q-type VGCC antibodies without clinical features of LEMS, but it is unknown if these patient have prolonged survival as well. In a substantial number of SCLC patients with paraneoplastic cerebellar degeneration (PCD) no other antineuronal antibodies than P/Q-type VGCC antibodies are detected, half of which have no clinical evidence for LEMS.

In this study we examined the frequency of P/Q-type VGCC antibodies and clinical features of LEMS in 148 consecutive patients with SCLC, and in 30 patients with PCD and SCLC, and studied their relation with survival.

Materials and methods
Consecutive patients with SCLC
From January 1990 to July 2001, a cohort of 148 consecutive Dutch patients with a SCLC, proven by cytology or histology, whose serum could be obtained at the time of diagnosis, were collected in the University Hospital Maastricht. Initial staging of these patients by a pulmonologist was based on physical examination, routine blood and chemistry profile, chest radiography, CT of the chest, ultrasound of the abdomen, radionuclide bone scan, fiberoptic bronchoscopy, MRI of the brain, and bone marrow aspirate and biopsy. Limited disease was defined as tumour confined to one hemithorax, including the mediastinum, the ipsilateral or contralateral scalene, and supraclavicular lymph nodes. In extensive disease tumour was found beyond these sites. A neurologist saw all patients at the time of diagnosis. Other neurological consultations or diagnostic tests took place as required. From each patient with SCLC a serum sample, obtained before chemotherapy, was kept frozen at -70°C until analysis. Only two patients were still alive at the time of the analysis. No patients had been lost to follow-up. Patients with PCD and SCLC
From the database in the Hospital Clinic, Barcelona, we selected Spanish patients with the final diagnosis of PCD with SCLC. These 30 patients presented with an isolated or clearly predominant cerebellar syndrome of unknown cause and developed SCLC.
Patients with paraneoplastic encephalomyelitis, which almost always have Hu antibodies, were excluded. The final outcome was obtained by interview with the referring physician. At the time of the analysis 20 patients were dead and seven had been lost the follow-up usually at the time of the tumour relapse (median one month, range 0-10 months).

**Diagnostic criteria for LEMS**
Diagnostic criteria for LEMS were the presence of P/Q-type VGCC antibodies, and clinical features (proximal muscle weakness, lowered tendon reflexes, dry mouth). Electromyography supported the diagnosis if it comprised a reduced resting compound muscle action potential amplitude that increased by >100% following high-frequency repetitive nerve stimulation (RNS) or maximal voluntary contraction.

**Antibody testing**
All patients were tested retrospectively for P/Q-type VGCC antibodies and Hu antibodies, and patient characteristics, staging and survival were compared between P/Q-type VGCC antibody positive patients with LEMS, P/Q-type VGCC antibody positive patients without LEMS and P/Q-type VGCC antibody negative patients.
To test for P/Q-type VGCC antibodies, an immunoprecipitation assay, using \[^{125}\text{I}\] -omega-conotoxin MVIIC, which binds to P/Q-type VGCC, was performed as described previously. Antibody titer was expressed as picomoles of \[^{125}\text{I}\] -toxin binding sites precipitated per litre of serum (pM). Sera were considered positive when the titre was greater than 50 pM (3 SD above the mean of the healthy controls). To test for Hu antibodies, Western blots using purified HuD fusion protein were performed as described previously.

**Statistical analysis**
Comparison of two qualitative variables was performed with the student t-test, the Pearson Chi-square or the Fisher’s exact test when appropriate. Survival was determined from the date of SCLC diagnosis to death. The relation of antibody status or LEMS and survival was analysed using Kaplan-Meier plots and the Log-rank test. Statistical analysis was performed using SPSS 10 (Statistical Product and Services Solutions, Chicago, IL).

**Results**
*Consecutive Dutch patients with SCLC (table)*
P/P-type VGCC antibodies were found in 10 patients (7%). Median antibody titre in these patients was 238.5 pM (range 76 to 1301 pM). In four of these patients (2.7% of total) clinical signs of LEMS were found; in three of them RNS was obtained, which
Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>P/Q-type VGCC antibody positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEMS</td>
<td>No LEMS</td>
</tr>
<tr>
<td>Number of patients</td>
<td>4</td>
</tr>
<tr>
<td>Mean age at diagnosis of SCLC ± SD (years)</td>
<td>50±8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male sex</td>
<td>3</td>
</tr>
<tr>
<td>Extensive disease</td>
<td>2</td>
</tr>
<tr>
<td>Response Complete&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
</tr>
<tr>
<td>No chemotherapy</td>
<td>0</td>
</tr>
<tr>
<td>Median survival and 95%CI (months)</td>
<td>24 (7-41)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hu antibody positive (%)</td>
<td>1</td>
</tr>
</tbody>
</table>

148 consecutive patients with SCLC.

30 patients with PCD and SCLC.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>5</th>
<th>9</th>
<th>16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at diagnosis of SCLC ± SD (years)</td>
<td>63±5</td>
<td>63±10</td>
<td>64±10</td>
</tr>
<tr>
<td>Male sex</td>
<td>5</td>
<td>9</td>
<td>14 (88%)</td>
</tr>
<tr>
<td>Extensive disease</td>
<td>0</td>
<td>3</td>
<td>7 (44%)</td>
</tr>
<tr>
<td>Median survival and 95%CI (months)</td>
<td>24 (0-50)</td>
<td>11 (6-16)</td>
<td>10 (2-18)</td>
</tr>
<tr>
<td>Hu antibody positive (%)</td>
<td>0</td>
<td>3</td>
<td>6 (38%)</td>
</tr>
</tbody>
</table>

VGCC - voltage gated calcium channel; LEMS - Lambert-Eaton myasthenic syndrome; PCD - paraneoplastic cerebellar degeneration.

<sup>a</sup> Compared to VGCC antibody negative patients p=0.006.

<sup>b</sup> Disappearance of all tumour masses detected in the initial staging procedures.

<sup>c</sup> Compared to VGCC antibody positive patients without LEMS p=0.02.

confirmed the diagnosis LEMS in one patient (>100% increase in compound muscle action potential amplitude after maximum voluntary contraction), but not in the other two patients in whom RNS was performed after chemotherapy. One other anti-VGCC antibody positive patient had cerebellar ataxia, but no signs of LEMS and no Hu antibodies. P/Q-type VGCC antibody positive patients with LEMS, but not those without LEMS, were significantly younger at diagnosis of SCLC than patients without antibodies (p=0.006). Although initial staging, response to therapy and survival in
antibody positive patients were not different from patients without antibodies, the four antibody positive patients with clinical evidence of LEMS had a median survival of 24.5 months (8, 24, 25 and 34 months), against only 10 months median survival in antibody negative patients (p=0.13), and only 7 months in antibody positive patients without LEMS (p=0.02) (Figure A). Hu antibody status had no relation with survival.

**Spanish patients with PCD and SCLC (table)**

In 30 patients with PCD and SCLC, 14 (47%) had P/Q-type VGCC antibodies. Of the antibody positive patients, five (36%) had clinical and RNS features consistent with LEMS. No differences in age at diagnosis of SCLC were found between the groups. The antibody positive patients with clinical evidence of LEMS had a median survival of 24 months against only 10 months in antibody negative patients (p=0.12), and only 11 months in antibody positive patients without LEMS (p=0.06) (Figure B).

**Discussion**

In this study, we found a remarkably long survival in patients with SCLC positive for P/Q-type VGCC antibodies, but only in patients with clinical signs of LEMS. This long survival was found in a cohort of consecutive patients with SCLC, as well as in an independent group of patients with SCLC and PCD.

In the group of 148 consecutive Dutch patients with SCLC, we found P/Q-type VGCC antibodies in 7% and Hu antibodies in 16% of the patients. This is in line with a study in 200 patients with SCLC (5% and 18.5%), that showed no relation of the presence of either antibody with prognosis, but did not take into account the presence of clinical features of LEMS.\(^9\) Our Dutch P/Q-type VGCC antibody positive patients with signs of LEMS indeed had a long survival. We studied a separate group of Spanish patients with SCLC and PCD, in which we could confirm these results, which seems to exclude a bias due to the retrospective nature of our study of the Dutch patients. In both groups of patients, over half of the patients with P/Q-type VGCC antibodies had no features of LEMS. Several studies found a low incidence of these antibodies (2-7%) in SCLC patients without paraneoplastic neurological syndromes.\(^6,10\) One study found a frequency of 18%, but used a lower upper limit of the normal range (2 S.D. instead of 3 S.D. above the mean for healthy controls).\(^5\) The presence of P/Q-type VGCC antibodies in SCLC patients without LEMS suggests that not all P/Q-type VGCC antibodies are pathogenic at the site of the neuromuscular synapse. The VGCC antibody immunoprecipitation assay will also detect antibodies binding intracellular epitopes, explaining why these antibodies did not result in prolonged survival. The survival curves suggest that the antibody positive SCLC patients without LEMS even have a shorter survival than antibody negative
Fig 1. Kaplan-Meier survival curves for A. 148 consecutive patients with small cell lung carcinoma (SCLC), and B. 30 patients with SCLC and paraneoplastic cerebellar degeneration. Curves are shown for P/Q-type voltage gated calcium channel (VGCC) antibody positive patients with clinical evidence of LEMS (VGCC+LEMS+), P/Q-type VGCC antibody positive patients without LEMS (VGCC+LEMS-) and P/Q-type VGCC antibody negative patients (VGCC-LEMS-). Censored cases of surviving patients or patients lost to follow-up are indicated by a square.
patients. Possibly, the aggressive tumour growth leads to increased tumour debris. The presentation of intracellular epitopes could result in a non-pathogenic anti-VGCC immune response.

The younger age at diagnosis of patients with LEMS in our group of consecutive patients with SCLC is in line with our recent epidemiological survey showing that the mean age at diagnosis of SCLC was 10 years lower in SCLC patients with concurrent LEMS, suggesting that patients who present with SCLC at an earlier age are additionally more prone to develop LEMS. Several but not all large series of SCLC patients report age as an independent prognostic factor, younger patients having favourable prognosis, but this is still a controversial issue. Possibly, SCLC patients with younger age generally have an anti-tumour immune response that is stronger, and therefore more frequently complicated by LEMS.

Acknowledgement
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References


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