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Anti-Hu-associated paraneoplastic encephalomyelitis: analysis of 200 patients

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Summary

We reviewed 200 patients with paraneoplastic encephalomyelitis (PEM) and anti-Hu antibodies to show possible clinical differences with respect to previous series, and to identify patient, tumour and treatment-related characteristics associated with neurological disability and survival. The median age of the 200 patients was 63 years (range 28–82 years) and 75% were men. The predominant neurological syndromes were sensory neuropathy (54%), cerebellar ataxia (10%), limbic encephalitis (9%) and multifocal involvement (11%). Sensorimotor neuropathies with predominant motor involvement were observed in only 4% of the patients. Pathological or X-ray evidence of a tumour was obtained in 167 patients (83%) and was a small-cell lung cancer (SCLC) in 74% of those with histological diagnosis. Coexistence of extrathoracic tumours with SCLC was rare (0.5%). Positive Hu immunoreactivity was observed in the extrathoracic tumours of six out of seven patients in whom autopsy or long-term follow-up ruled out a coexisting SCLC. PEM preceded the diagnosis of the tumour in 71% of patients (mean delay ± SD 6.5 ± 7.0 months; range 0.1–47 months). In the 24 patients in whom the tumour diagnosis was the initial event, PEM predicted the progression or relapse of the tumour in 87% of them. No tumour was found in 33 patients, including four who had a post-mortem study and four with >5 years of follow-up. In a logistic regression analysis, treatment of the tumour, associated or not with immunotherapy, was an independent predictor of improvement/stabilization of PEM [odds ratio 4.56; 95% confidence interval (CI) 1.62–12.86]. Cox multivariate analysis indicated that the variables independently associated with mortality were: age >60 years [relative risk (RR) 1.49; 95% CI 1.05–2.12], Rankin score at diagnosis >3 (RR 1.60; 95% CI 1.12–2.28), more than one area of the nervous system affected (RR 1.61; 95% CI 1.08–2.40), and absence of treatment (RR 2.56; 95% CI 1.76–3.71). We conclude that, unlike previous series, the majority of our patients were male, and there was a low occurrence of predominantly motor neuropathies and extrathoracic tumours coexisting with SCLC. When the diagnosed extrathoracic tumour expresses Hu antigens, further tests to rule out a coexisting SCLC are probably unnecessary. Finally, the predictors of mortality and PEM evolution found in the study may be important in the design of future therapeutic protocols, and emphasize the importance of early diagnosis and treatment of the underlying tumour.

Keywords: autoantibodies; encephalomyelitis; paraneoplastic; sensory neuropathy; small cell lung cancer

Abbreviations: LEMS = Lambert–Eaton myasthenic syndrome; PEM = paraneoplastic encephalomyelitis; SCLC = small cell lung cancer

Introduction

Paraneoplastic encephalomyelitis (PEM), one of the most frequent remote effects of cancer, is pathologically characterized by neuronal loss and inflammatory infiltrates in particular areas of the nervous system (Henson, 1982). The location and severity of the neuronal loss predicts the clinical symptoms of the patient that may be confined to one area of the nervous system or, more frequently, involve over time, multiple areas (Table 1). The clinical onset is subacute,

Table 1 *Paraneoplastic encephalomyelitis: clinical and pathological correlation*

Pathological target	Clinical syndrome
Hippocampus	Limbic encephalitis
Brainstem	Brainstem encephalitis
Cerebellum	Cerebellar syndrome
Spinal cord	Motor neuronopathy
Dorsal root ganglia	Sensory neuronopathy
Myenteric plexus	Intestinal pseudo-obstruction
Combination of the above	Encephalomyelitis

usually causes a severe neurological dysfuction, and antedates the diagnosis of a small cell lung cancer (SCLC) in >70% of cases.

The majority of patients with PEM harbour in the serum and CSF an antineuronal antibody, anti-Hu (Graus *et al*., 1985, 1986), that recognizes a family of RNA-binding proteins (HuD, HuC, Hel-N1 and Hel-N2) expressed in the nuclei of neurones and SCLC cells (Szabo *et al*., 1991; King and Dropcho, 1996). The Hu antigens have a crucial role in the development and maintenance of the neuronal phenotype, but their function in the tumour cells is unknown. There is no evidence that the anti-Hu antibodies are the cause of the neuronal damage in PEM (Sillevis-Smitt *et al.*, 1995; Carpentier *et al.*, 1998), but they represent a useful diagnostic marker (Molinuevo *et al*., 1998) and probably are part of a more complex immune response against Hu antigens that is initially driven to control tumour growth but misdirected to cause neurological dysfuction (Posner and Dalmau, 1997).

Two large series described the clinical features of anti-Hu-associated patients with PEM (Dalmau *et al.*, 1992*a*; Lucchinetti *et al.*, 1998). They showed some discordance in the frequency of sensorimotor neuropathy and autonomic dysfunction, and revealed the number of patients with an SCLC coexisting with other tumour types. Moreover, a detailed description of the severity of the neurological dysfunction, treatments used and response of PEM was not provided. In the present study, we analysed a series of 200 patients with PEM and anti-Hu antibodies to show possible clinical features with respect to previous series and to identify possible patient, tumour and treatment-related characteristics associated with neurological disability and survival.

Methods

Patients

From 1987, all patients with anti-Hu antibodies diagnosed in the two participating laboratories were included in a database that contains information on the neurological symptoms, severity of neurological dysfunction, delay in the neurological diagnosis, tumour diagnosis and staging, treatments received and outcome of both the PEM and tumour. The information was obtained from forms filled out by the referring neurologists, telephone interviews and review of the clinical records. Sixty-four (32%) patients were seen personally by at least one of the authors.

All patients included in the study presented serum anti-Hu antibodies with a titre of 1 : 1000 or higher (usually ⩾1 : 5000), detected by immunohistochemistry on frozen sections of human or rat brain and confirmed by immunoblot of neuronal nuclei extracts or HuD fusion protein. All techniques have been described previously (Graus *et al.*, 1986, 1997). The cut-off of 1 : 1000 was chosen because this titre was almost always associated with PEM. SCLC patients without PEM rarely presented high anti-Hu titres, whereas a lower titre may be seen in up to 16% of them (Dalmau *et al.*, 1990; Graus *et al.*, 1997).

For the purpose of the study, 18 anti-Hu positive patients were excluded because the clinical information was very incomplete. The clinical symptoms (at presentation and those predominant at diagnosis) of the remaining 200 patients were coded into six syndromes: cortical, brainstem, cerebellar, sensory neuropathy, sensorimotor neuropathy and autonomic dysfunction. We always tried to ascertain a single predominant syndrome on the basis of the information received or personally obtained. When more that one syndrome was equally predominant, the clinical data were entered as multifocal. Patients with features of sensory and motor neuropathy were coded as sensory neuropathy if the patient presented typical and predominant features of large fibre sensory neuropathy and the motor symptoms were mild or only manifested themselves in the electromyographic examination. Annual meetings of the two laboratories were held to assure a uniform criteria for the evaluation of the written forms and introduction of the information in the database.

The tumour response was assessed by the patient's oncologist at the end of treatment. Treatment of PEM included antineoplastic therapy of the tumour, immunotherapy or both (Graus *et al.*, 1992; Uchuya *et al.*, 1996; Keime-Guibert *et al.*, 2000). The neurological disability was evaluated by a modified Rankin scale (Graus *et al.*, 1992). In this scale, 0 = asymptomatic patient; 1 = symptoms do not interfere with lifestyle; 2 = symptoms lead to some restriction of lifestyle but do not prevent totally independent existence; 3 = symptoms significantly interfere with lifestyle or prevent totally independent existence; 4 = symptoms clearly prevent independent existence, although patient does not need constant attention day and night; 5 = severe disability, with patient totally dependent and requiring constant attention day and night; 6 = death due to the PEM. Patients with an indolent PEM (Graus *et al.*, 1994) were defined as those who had a Rankin score <3 at the diagnosis of anti-Hu and whose symptoms remained stable without treatment for at least 12 months.

Hu immunoreactivity of extrathoracic tumours

Paraffin sections of extrathoracic tumours from patients of this series were deparaffinized in xylene, rehydrated in

alcohol, washed in tap water and heated for 2 min in a pressure cooking oven in 0.1 M sodium citrate buffer (pH 6.0) (Giometto *et al.*, 1996). After inhibition of endogenous peroxidase with 0.3% hydrogen peroxide in PBS (phosphate-buffered saline) for 15 min, sections were sequentially incubated with undiluted normal human serum or a human serum with high titres of anti-Hu antibodies for 2 h at room temperature; biotinylated anti-Hu IgG obtained as described previously (Furneaux *et al.*, 1990) overnight at 4°C. The Vectastain Elite ABC complex (Vector Labs, Irvine, Calif., USA) was incubated for 30 min. The reaction was developed with 0.05% diaminobenzidine and 0.01% hydrogen peroxide in PBS with 0.5% Triton X-100 (Gultekin *et al.*, 1998).

Tumours that immunoreacted with the biotinylated anti-Hu IgG were considered to express the Hu antigen if the immunoreactivity was abolished by preincubation with a human serum with high titres of anti-Hu antibodies. A section of SCLC or human cortex was used as positive control.

Six tumours that showed Hu immunoreactivity were also analysed with synaptophysin and chromogranin antibodies (Dako, Denmark) using a conventional avidin–biotin immunoperoxidase technique.

Statistical analysis

The date of PEM diagnosis was that of the positive detection of serum anti-Hu antibodies. Survival was determined from onset of neurological symptoms to death or last visit. As of October 1999, 25 patients were alive with a median follow-up duration of 35 months (range 14–110 months). Fifteen patients were lost after a median follow-up of 14 months (range 1.0–102 months). All but three patients were lost when their cancer was at a terminal stage (eight patients) or had severe neurological dysfunction (four). Survival analysis was based on the Kaplan–Meier test.

The Spearmann correlation was used to analyse the relationship between the Rankin score at diagnosis of PEM and the delay between onset of PEM and diagnosis.

A Cox regression analysis (Cox, 1972) was used to estimate the influence of important variables on survival times in those patients with initial presentation of PEM. The variables included in the model were: sex, age (>60 versus $\leqslant 60$ years), presence of tumour, Rankin score at diagnosis of PEM (>3 versus $\leqslant 3$), presence of CNS involvement, number of areas of the nervous system affected (1 versus >1), and treatment (no treatment versus any kind of immunotherapy or antineoplastic treatment).

Odds ratios were used to estimate the magnitude of associations between pre-treatment variables (see above), type of treatment (no treatment, immunotherapy, oncological treatment $\pm$ immunotherapy) and stabilization/improvement of PEM. Patients excluded from the analysis included 24 in whom the diagnosis of cancer preceded the PEM, 17 who had a stabilization of PEM <6 months due to death not related to PEM, and two with no clinical information. Because not all patients were treated, a patient was considered neurologically improved or deteriorated if there was a change of at least 1 point in the Rankin scale measured at the onset of the treatment or at the time of the PEM diagnosis (for non-treated patients) and the score of the last visit. Therefore, in those patients who received sequential treatment with immunotherapy and in whom their tumour was treated later, the final clinical outcome reflected the combined effect of both treatments on PEM evolution. The Rankin score at diagnosis and onset of treatment was the same in all but two of the patients who were treated. The adjusted odds ratio and 95% CI (confidence interval) were calculated by logistic regression (Hosmer and Lemeshow, 1989). The SPSS 9.0 Statistical Software was used for the statistical analysis.

Results

The median age of the 200 patients was 63 years (range 28–82 years); 151 were men and 49 women (Table 2). One-hundred-and-nine patients were Spanish, 84 French and seven from other European countries. No differences were observed in age, predominant neurological syndrome at diagnosis of PEM, delay in the diagnosis of PEM, severity of the neurological disability, tumour type distribution, number of patients treated, and response to treatment between Spanish and French patients. However, the frequency of men was significantly higher among Spanish (85%) than French (67%) patients ($P = 0.002$).

Histological or X-ray evidence of a tumour was not found in 33 (16.5%) patients. The clinical characteristics of these patients were not different from those with cancer, except that in the non-tumour group there was a higher proportion of women (39 versus 22% of total patient group; $P = 0.02$). Seventeen of the 33 patients without tumour had a short survival (<1 year) and autopsy was carried out in only three of them. Twelve of the 33 patients had a follow-up between 12 and 36 months. Five of them died and a post-mortem study was done on one. The other seven patients are alive (five) or lost (two). One of the living patients is a 28-year-old woman with systemic lupus erithematosus and a sensorimotor neuropathy that improved spontaneously. She is tumour-free after 33 months of follow-up. Two men were treated with chemotherapy alone, assuming they had an occult SCLC based on the presence of anti-Hu antibodies and history of smoking. One them is alive after 27 months of follow-up and the second patient died from complications of the PEM 22 months after diagnosis.

Four of the 33 patients without tumour had a follow-up of >5 years. Three patients had sensory neuropathy, two were women whose clinical features were reported previously [Graus *et al.*, 1994 (Patient 3); Molinuevo *et al.*, 1998]. The third patient was a 54-year-old man with a severe sensory neuropathy who died 10 years later without evidence of tumour; autopsy was not performed. The last patient was a 61-year-old man with cerebellar ataxia and opsoclonus who was lost after a follow-up of 70 months. The initial

bronchoscophy showed a severe pre-malignant bronchial displasia, but several months later two subsequent bronchoscopies disclosed a normal bronchial mucosa.

Features relating to the cancer

Histological confirmation of cancer was obtained in 149 (74.5%) patients. Eighteen (9%) showed radiological evidence of lung cancer, almost always enlarged mediastinal lymph nodes, but histological diagnosis could not be obtained. PEM led to the diagnosis of the tumour (X-ray or histological) in 143 (71.5%) patients after a mean delay (±SD) of 6.5 (±7.0) months (range 0.1–47 months). Tumour staging was known in 121 of the 128 patients in whom PEM lead to diagnosis of the tumour and histological confirmation was obtained, and systemic metastasis was found in 30 (24.8%) of them, with no differences between SCLC and other histological types. Tumour was discovered at the post-mortem study in eight patients.

The tumour antedated the diagnosis of PEM in 24 patients. PEM appeared in the first 6 months after tumour diagnosis in 15 patients and the cancer progressed or relapsed in all of them. In the other nine patients, PEM appeared when the tumour was in remission (median time 12 months; range 9–67 months), and PEM predicted the tumour relapse in six.

The tumour was located in the lung in 144 patients, including 18 with X-ray evidence only, followed by location in the prostate and gastrointestinal system (Table 3). The most frequent histological type of tumour in the lung and other locations was small cell cancer (114 patients), followed by undifferentiated large cell tumour, adenocarcinoma or epidermoid carcinoma (28 patients), malignant neuroendocrine tumour (seven) and ovarian dysgerminoma (one). Three patients presented another tumour [lung adenocarcinoma (two) and breast cancer] coexisting with an SCLC.

Hu immunoreactivity was studied in 14 of the 20 extrathoracic non-small cell tumours and in the breast cancer of the patient who also had SCLC. Two of the 14 tumours [rectum, previously reported (Toffol *et al.*, 1997), and gall bladder] available for analysis had a neuroendocrine histology and both showed strong immunoreactivity with anti-Hu antibodies as well as with other neuroendocrine markers (synaptophysin and chromogranin). Ten (83%) of the remaining 12 tumours [breast (three), ovary (two), bladder (two), prostate (one), oesophagus (one), colon (one)] showed Hu immunoreactivity, whereas two (prostate,

Table 2 *Clinical characteristics of 200 anti-Hu-associated PEM patients*

Characteristics	(%)
Median age, years (range)	63 (28–82)
Males, *n*	151 (75.5)
Predominant syndrome at diagnosis, *n*	
Sensory neuropathy*	108 (54.0)
Cerebellar	21 (10.5)
Cortical encephalitis†	20 (10.0)
Brainstem	12 (6.0)
Sensorimotor neuropathy	9 (4.5)
Dysautonomia‡	8 (4.0)
Multifocal	22 (11.0)
Rankin score at diagnosis	
1–3	93 (46.7)
4–5	106 (53.3)
Unknown	1
Mean delay ± SD to diagnosis anti-Hu, months (range)	6.8 ± 7.8 (0.2–47)
Mean delay ± SD to tumour diagnosis, months (range)	6.5 ± 7.0 (0.1–47)
Tumour type, *n*	
Small cell lung carcinoma	111 (55.5)
Other	38 (19.0)
X-ray	18 (9.0)
No tumour	33 (16.5)
Median survival, months (range)	11.8 (0.7–121.4)
Probability of survival, %	
12 month	47
36 months	20
60 months	12
Cause of death, *n*	
Paraneoplastic encephalomyelitis	76 (60.3)
Tumour related	50 (39.7)
Unknown	34

*Including patients with clinical or neurographic evidence of mild motor weakness or dysautonomia.
†All but three patients had limbic encephalitis. ‡Chronic pseudo-obstruction in seven patients.

unknown origin) were negative. The breast cancer of the patient with coexisting SCLC did not express Hu antigens. Hu immunoreactivity was present in >50% of the tumour cells in eight of the 10 tumours (Fig. 1), but in two (prostate, ovarian dysgerminoma) only a minority of cells were positive (Fig. 2A and B). Seven of these 12 patients whose extrathoracic tumour was analysed had an autopsy or a follow-up of 3 years or longer without evidence of SCLC and six showed a positive Hu immunoreactivity.

Hu immunoreactivity was more frequent and widespread than that observed with synaptophysin or chromogranin in the six Hu-positive tumours that were tested with all the markers. Four tumours (prostate, breast, colon, ovarian dysgerminoma) showed widespread Hu labelling, whereas the neuroendocrine markers were negative, and in the other two (ovary, bladder) the number of Hu-positive cells was higher than those that immunoreacted with synaptophysin or chromogranin.

Table 3 *Location and histologic diagnosis of neoplasms in 167 patients with anti-Hu-associated PEM*

Location	*n* (%)
Lung	144 (85.6)
SCLC*	111
Non-SCLC	15 (four neuroendocrine)
X-ray	18
Extrathoracic	23 (14.4)
Prostate	6 (one small cell)
Gastrointestinal†	6 (one small cell, three neuroendocrine)
Breast	3
Bladder	2
Pancreas	2 (one small cell)
Ovary	2
Unkown origin	2

*Three SCLC coexisted with lung (two) or breast (one) adenocarcinomas. †Stomach (two), oesophagous (one), gall bladder (one), colon (one) and rectum (one).

Features relating to the PEM

The predominant neurological syndrome at diagnosis of PEM is shown in Table 2. Neurological dysfuncion was confined to one area of the nervous system in 60 (30%) patients [sensory neuropathy (48), cerebellar ataxia (four), limbic encephalitis (four), brainstem encephalitis (two), intestinal pseudo-obstruction (one) and parietal encephalitis (one) (Shavit *et al.*, 1999)]. The other patients had evidence of multifocal involvement. A predominant neurological syndrome was identified in 118 of them, whereas in 22 more than one syndrome predominated during the clinical evolution.

Sensory neuropathy was the most frequent predominant syndrome at diagnosis in 108 (54%) patients. The clinical features were those of large fibre sensory neuropathy (Denny-Brown, 1948; Horwich *et al.*, 1977). However, pure sensory neuropathy was observed in only 48 (24%) patients. The other 60 patients had symptoms of involvement of other areas of the nervous system. CNS involvement was found in 27 patients. Sensory and motor involvement in the absence of symptoms of CNS dysfunction was observed in 14 patients. These patients had mild distal muscle weakness that was

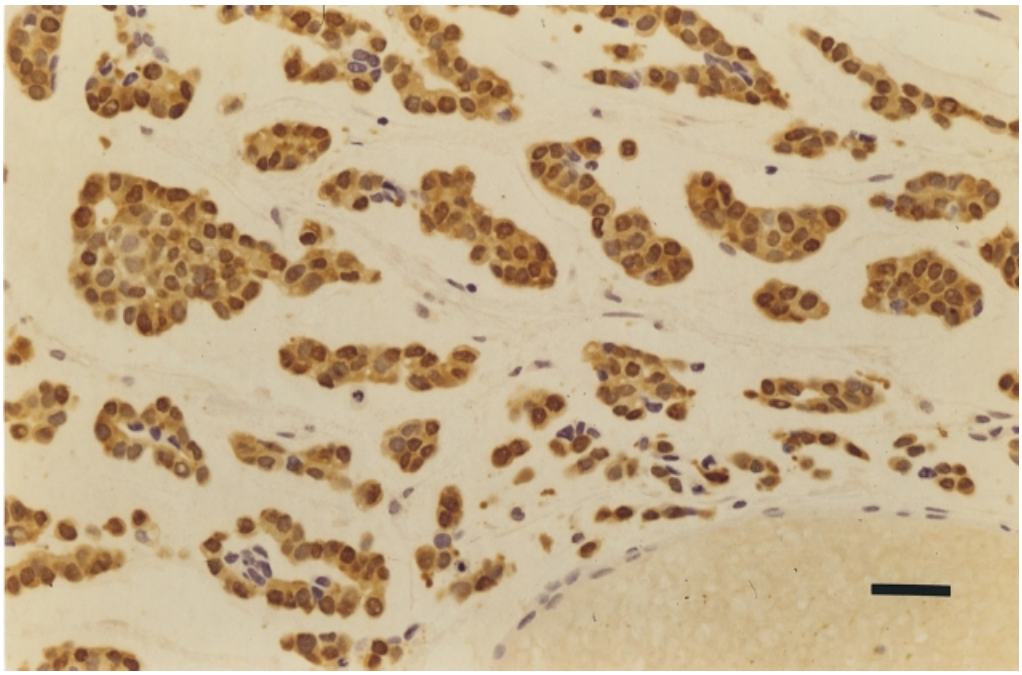

Fig. 1 Breast cancer of a non-smoking woman with no evidence of SCLC at post-mortem. The tumour was immunoreacted with biotinylated anti-Hu IgG. Tumour cells show Hu labelling. Slight counterstain with haematoxylin. Bar = 120 μm.

overshadowed by the severity of the sensory deficit. Autonomic system involvement was detected in 19 patients with predominant sensory neuropathy (four also had motor weakness). Gastrointestinal dysmotility with persistent constipation, vomiting and X-ray evidence of gastric, small bowel or colon dilatation was the most frequent complaint in 13 patients (Lucchinetti *et al*., 1998). The other patients complained of orthostatic hypotension of urinary dysfunction.

Sensorimotor neuropathy was seen in nine (4.5%) patients. All of them had muscle weakness that was at least as severe as the sensory symptoms. None had a syndrome that could be confused with motor neurone disease. Four of them developed severe, generalized weakness that progressed to respiratory insufficiency and required mechanical ventilation (Graus *et al.*, 1987). The other four patients showed a clinical picture that was compatible with a chronic polyneuritis.

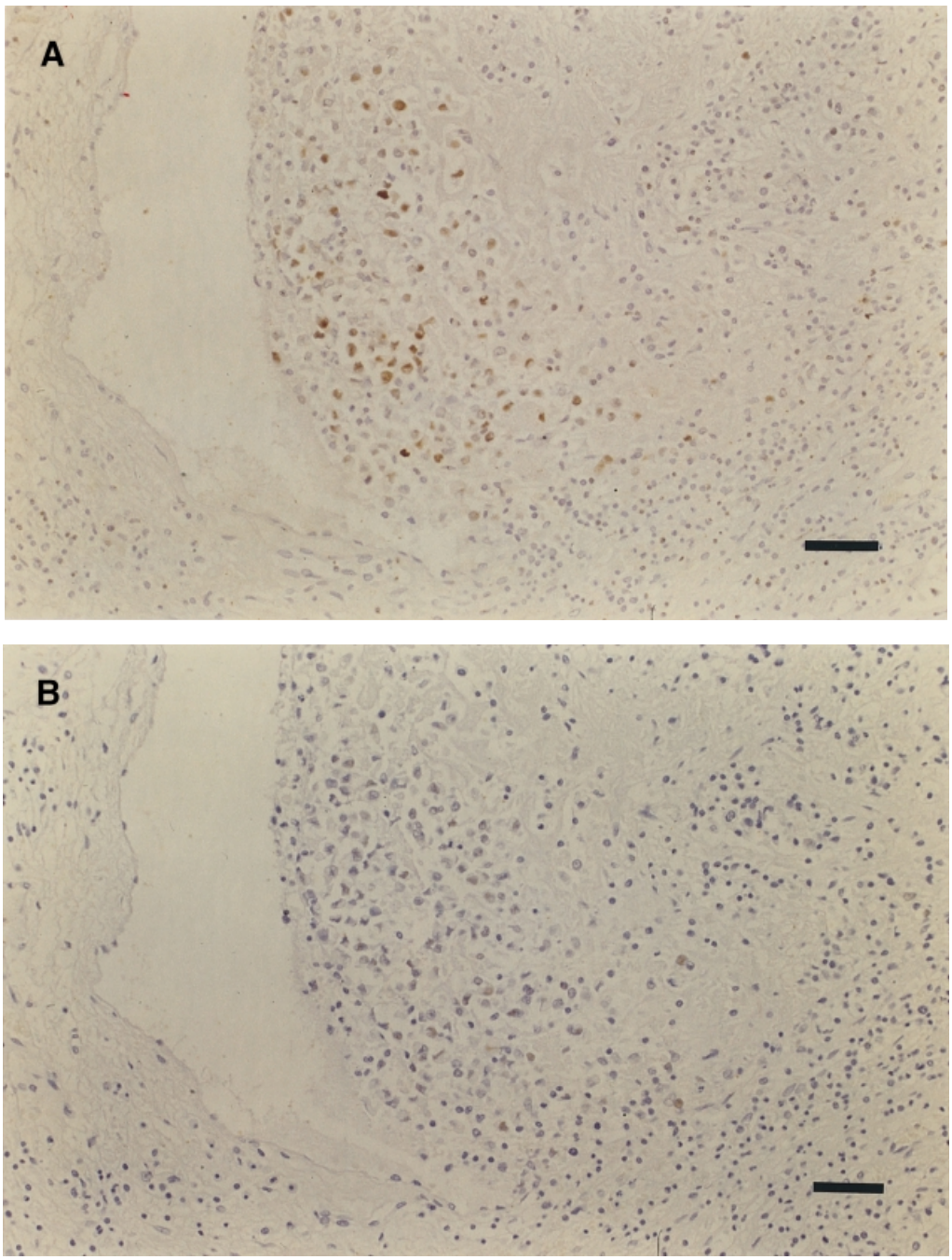

Fig. 2 Serial sections of ovarian dysgerminoma. (**A**) The section was incubated with biotinylated anti-Hu IgG. A few tumour cells were positive. (**B**) The positive immunoreactivity was abolished by pre-incubation of the section with a serum with high titres of anti-Hu antibodies. Slight counterstain with haematoxylin. Bar = 300 μm.

Finally, one patient presented with clinical and neurophysiological involvement compatible with damage of the lumbosacral nerve roots.

Predominant CNS symptoms were found in 75 (32.5%) patients, including those who presented with simultaneous involvement of multiple areas of the nervous system. Cerebellar and brainstem disorders, sometimes mimicking cerebrovascular disease, were the most common presentations followed by limbic encephalitis (Alamowitch *et al.*, 1997; Mason *et al.*, 1997). Three patients presented with a syndrome of epilepsia partialis continua due to a focal involvement of the cortical sensorimotor area (Shavit *et al.*, 1999).

PEM usually caused a severe neurological dysfuction and 106 patients had a Rankin score of >3 at diagnosis. There was a correlation between the Rankin score and delay in the diagnosis of anti-Hu antibodies ($r = -0.223$; $P = 0.002$). The diagnostic delay was longer in those patients who presented with a more benign neurological syndrome (Fig. 3). Ten (5%) patients had an indolent PEM (Graus *et al.*, 1994). They presented with sensory neuropathy (six patients), sensorimotor neuropathy, cerebellar ataxia, limbic encephalitis and multifocal involvement. The outcome of PEM was bad. Sixty-one patients were not treated and only one improved. She was an atypical patient (described above) with systemic lupus erythematosus and sensorimotor neuropathy. Antineoplastic treatment was given to 82% of the patients with histologically proved tumour diagnosed after PEM. Ninety patients received some form of immunotherapy associated or not with treatment of the tumour. Immunotherapies included: intravenous immunoglobulins (0.5 g/kg/day for 5 days) in 37 patients, the same treatment associated with cyclophosphamide (600 mg/m^2 for 1 day) or methylprednisolone (1 g/day for 3 days) in 19, methylprednisolone (1 g/day for 3 days) in 18 and plasmapheresis in 13. One patient was treated with cyclophosphamide alone, and one had immunoadsorption with protein A column and photopheresis. The clinical features of the nine patients who improved with treatment are summarized in Table 4. Four patients improved after immunotherapy and five after antineoplastic therapy with (four patients) or without (one) concomitant immunotherapy.

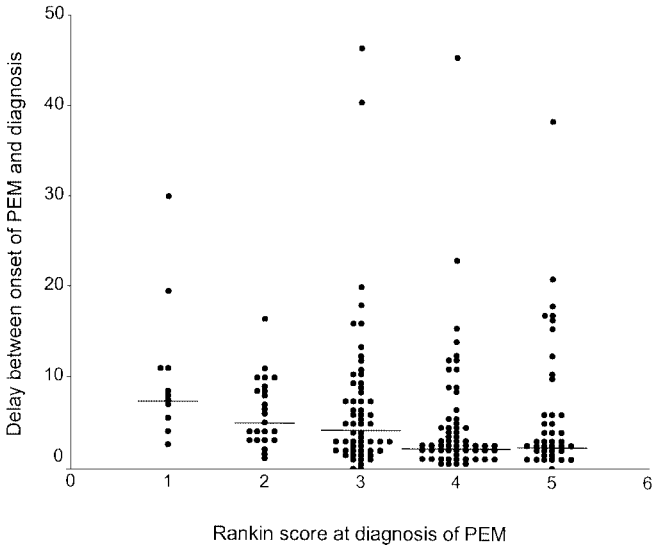

Fig. 3 Relationship between Rankin score at PEM diagnosis and delay between onset of PEM and diagnosis of anti-Hu antibodies. Horizontal bars represent the median for each Rankin score.

Evolution of PEM according to treatment with immunotherapy alone or antineoplastic therapy with or without immunotherapy was compared with that of patients who were not treated (see Methods). Improvement or stabilization of PEM was observed in 37.5% of the 80 patients treated with antineoplastic therapy with or without immunotherapy, 20.6% of 34 patients treated with immunotherapy and 11.6% of 43 untreated patients. After multivariate adjustment for pre-treatment variables (age, sex, Rankin score at diagnosis, predominant involvement of the CNS, number of areas of the nervous system involved), the probability of at least stabilization of PEM at the last visit was significantly increased in the group of patients treated with antineoplastic therapy with or without immunotherapy (odds ratio 4.56; 95% CI 1.62–12.86).

The median survival of the 200 patients was 11.8 months, with a 3-year actuarial survival of 20%. To assess the independent prognostic value of treatment of PEM and different pre-treatment variables (see Methods) for survival, a Cox regression analysis was used. Table 5 shows that age, Rankin score at diagnosis of PEM, number of areas of the nervous system affected and treatment were independently associated with survival.

Discussion

The present analysis of 200 patients with anti-Hu-associated PEM showed some relevant differences between our results and those of previous studies (Dalmau *et al.*, 1992*a*; Lucchinetti *et al.*, 1998), described new clinical features relating to PEM and cancer and identified prognostic factors related to treatment response and survival.

Unlike previous American series (Dalmau *et al.*, 1992*a*; Lucchinetti *et al.*, 1998), the frequency of women in this study was much lower, with significant differences between Spanish (15%) and French (33%) patients. The most likely explanation for this feature is the different incidence of SCLC among women in these countries. The frequency of women in large series of SCLC is up to 42% in the US (Turrisi *et al.*, 1999), whereas in France it is 13% (Arriagada *et al.*, 1993) and in Spain just 5% (Graus *et al.*, 1997). These data show that the proportion of women in studies of anti-Hu-associated PEM is correlated to the incidence of women in SCLC studies and as previously suspected, women are more likely to develop PEM if they have SCLC.

The actual incidence of PEM patients without tumour is unknown because most patients die without autopsy and a post-mortem study can easily miss a microscopic tumour. Long-term follow-up is probably the best evidence that a neurological paraneoplastic syndrome may occur in absence

Table 4 *Clinical characteristics of PEM patients who improved after treatment*

Patient	Age (years)/ sex	Syndrome	Rankin score at diagnosis	Tumour	Tumour treatment	Tumour response	Immunotherapy	Follow-up (months)
1	71/M	Sensory neuropathy	4	SCLC	Yes	CR	IVIG	44
2	73/M	Sensory neuropathy	4	SCLC	Yes	CR	IVIG	64+
3	43/F	Sensory neuropathy	2	No	No	NA	IVIG	82+
4	61/M	Multifocal	2	SCLC	No*	NA	IVIG + steroids	8
5	69/M	Cerebellar ataxia	5	SCLC	Yes	CR	No	11
6	53/M	Cerebellar ataxia	5	X-ray	No	NA	IVIG + steroids	9
7	56/F	Parietal encephalitis	3	SCLC	No*	NA	cyclophosphamide	36+
8	49/M	Sensory neuropathy	4	Non-SCLC	Yes	CR	IVIG	14+
9	63/M	Sensory neuropathy	4	SCLC	Yes	CR	IVIG	11+

M = male; F = female; NA = not applicable; IVIG = intravenous immunoglobulins. *PEM appeared when tumour was in remission.

Table 5 *Predictors of mortality according to Cox multivariate analysis in 174 patients who presented with paraneoplastic encephalomyelitis**

Variable	Relative risk	95% CI
Age >60 (years)	1.49	1.05–2.12
Rankin score at diagnosis >3	1.60	1.12–2.28
More than one areas of nervous system involved	1.61	1.08–2.40
Absence of treatment	2.56	1.76–3.71

*Two patients were excluded because some variables were missing.

of tumour. In Lambert–Eaton myasthenic syndrome (LEMS), SCLC almost always appears in the first 3 years, but >25% of LEMS patients never develop cancer after a follow-up of >4 years (O'Neill *et al.*, 1988; Tim *et al.*, 2000). By contrast, in this study only four (2%) patients did not develop cancer after >4 years and in one, the possibility of spontaneous remission of the tumour could not be dismissed (Darnell and DeAngelis, 1993). This observation may be relevant for the management of these patients. The outcome of SCLC in PEM patients once the tumour is radiologically visible is poor, with a 3 year actuarial survival of 30% (Keime-Guibert *et al.*, 1999). In addition, effective treatment of the tumour is an independent predictor for stabilization of PEM (Keime-Guibert *et al.*, 1999). These data, coupled with the high probability of anti-Hu-associated PEM patients harbouring a tumour, support the design of therapeutic protocols with chemotherapy in patients with PEM when the tumour is not found at the initial evaluation and the risk of SCLC is greatest (age >50 years and with a smoking habit).

Previous work suggested that when a tumour other than SCLC was discovered in PEM patients the possibility of a concomitant SCLC was high and appropriate studies should be made to rule out this tumour (Lucchinetti *et al.*, 1998). However, the coexistence of other tumours with SCLC was rare in the present series. Extrathoracic tumours from patients without PEM almost never express Hu antigens (Damau *et al.*, 1992*b*) so we hypothesized that a positive Hu immunoreactivity by the extrathoracic tumour would support that the tumour was responsible for the PEM. We observed that the majority (83%) of extrathoracic tumours indeed expressed Hu immunoreactivity. Seven of the patients with them had an autopsy or a follow-up of at least 3 years without evidence of a concomitant SCLC, and the tumour was Hu positive in six. Therefore, a positive Hu immunoreactivity strongly supports the tumour found being responsible for PEM, and further studies to discover an SCLC would not be necessary in these patients. We observed that the Hu expression may be restricted to a few cells so the possibility of a false negative result should be expected if the analysis is done on small biopsies, as is the case of prostate cancer.

The predominant neurological symptoms of PEM in this study are similar to those of previous studies (Dalmau *et al.*, 1992*a*; Lucchinetti *et al.*, 1998), except for the lower number of patients with peripheral sensorimotor weakness. This discrepancy is probably due to the way we have classified PEM symptoms. We defined patients with minor motor involvement as sensory rather than sensorimotor neuropathy to emphasize that, in them, the sensory symptoms were much more relevant than the motor weakness. The sensory symptoms in PEM patients are usually due to a lesion in the dorsal root ganglia rather than in the peripheral nerve, as occurs in the common sensorimotor neuropathies (Denny-Brown, 1948). The cause of the motor weakness in PEM patients is unclear and probably not uniform. There are autopsy studies that demonstrate a loss of motor neurones in the anterior horn of the spinal cord (Henson *et al.*, 1965; Graus *et al.*, 1987) or involvement of peripheral nerves with inflammatory infiltrates and demyelination (Younger *et al.*, 1994; Antoine *et al.*, 1998; Eggers *et al.*, 1998). The role of these T cell infiltrates, which do not express Hu antigens, in the peripheral nerves or muscles is unknown. The inflammatory infiltrates could indicate a concurrent paraneoplastic

syndrome (Oh *et al*., 1991), as occurs with paraneoplastic cerebellar degeneration and LEMS (Mason *et al.*, 1997), triggered by antigens different from Hu (Antoine *et al.*, 2001).

The prognosis of PEM remains poor in terms of survival and neurological disability. Similar to SCLC patients without PEM (Spiegelman *et al.*, 1989), age and functional status, measured in our patients by the Rankin score, provided independent prognostic factors for survival. We included in the multivariate analysis only the patients who presented with PEM because this group includes the majority of patients with PEM (88%) and is the natural target of future prospective, ideally randomized, therapeutic protocols that should consider the influence of the pre-treatment prognostic factors identified in the present study.

Up to 53% of patients were severely disabled at the time of diagnosis and only a minority (5%) had a benign indolent course (Graus *et al.*, 1994). In the multivariate analysis after adjusting for potential confounding variables, treatment of the tumour with or without concomitant immunotherapy was an independent predictor of at least stabilization of the neurological dysfunction for 6 months or more. At present, early diagnosis and treatment of the tumour gives the best chance of being able to stabilize the disease (Keime-Guibert *et al.*, 1999). We observed an inverse correlation between severity of PEM at diagnosis and delay between onset of PEM and diagnosis of anti-Hu antibodies. Neurologists must be aware that some patients may present with a more insidious form of PEM and a chronic evolution of the neurological symptoms does not rule out the possibility of PEM. Moreover, patients who were severely disabled at the time of diagnosis (Rankin score >3) had a median diagnostic delay of 3 months, suggesting PEM diagnosis should be pursued more aggressively. PET may detect lung cancer, particularly mediastinal involvement, the most common situation in PEM patients (Chartrand-Lefebvre *et al.*, 1998), when other tests are negative (Pieterman *et al*., 2000) and should be carried out in PEM patients when cancer is not found by conventional radiological procedures (Antoine *et al*., 2000).

Although immunotherapy alone is probably not effective in the majority of patients (Graus *et al.*, 1992; Uchuya *et al*., 1996; Keime-Guibert *et al*., 2000), previous case reports (reviewed by Oh *et al*., 1997; Das *et al.*, 1999) and four patients in this study improved with different immunotherapies. Byrne *et al.* (1997) reported two patients with PEM and anti-Hu antibodies who made a spontaneous improvement. Patient 1, who also had a probable regression of the lung cancer and PEM, made a great improvement after resection of the already remitting lung nodules that showed infiltrates of lymphocytes and plasma cells, but no cancer in the pathological examination. In addition, PEM probably has an autoimmune pathogenesis (Benyahia *et al.*, 1999). Therefore, a trial with immunotherapy should be considered in PEM patients when antineoplastic treatment is not possible because a tumour is not found or when PEM appears during or after tumour treatment. No specific immunotherapy treatment can be recommended on the basis of this and previous studies. Ideally, all PEM patients should be offered the possibility of inclusion in therapeutic protocols.

We conclude that, unlike in previous studies, the majority of our patients were male and there was a low occurrence of predominantly motor neuropathies and extrathoracic tumours coexisting with SCLC. When an extrathoracic tumour is found and Hu expression is demonstrated, further tests to rule out a coexisting SCLC are probably are not necessary. Finally, the predictors of mortality and PEM evolution found in the study may be important in the design of future therapeutic protocols and emphasize the importance of early diagnosis and treatment of the underlying tumour.

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References

Alamowitch S, Graus F, Uchuya M, Reñé R, Bescansa E, Delattre JY. Limbic encephalitis and small cell lung cancer. Clinical and immunological features. Brain 1997; 120: 923–8.

Antoine JC, Mosnier JF, Honnorat J, Convers P, Absi L, Lapras J, et al. Paraneoplastic demyelinating neuropathy, subacute sensory neuropathy, and anti-Hu antibodies: clinicopathological study of an autopsy case. Muscle Nerve 1998; 21: 850–7.

Antoine JC, Cinotti L, Tilikete C, Bouhour F, Camdessanché JP, Confavreux C, et al. [^{18}F]fluorodeoxyglucose positron emission tomography in the diagnosis of cancer in patients with paraneoplastic neurological syndrome and anti-Hu antibodies. Ann Neurol 2000; 48: 105–8.

Antoine JC, Honnorat J, Camdessanché JP, Magistris M, Absi L, Mosnier JF, et al. Paraneoplastic anti-CV2 antibodies react with peripheral nerve and are associated with a mixed axonal and demyelinating peripheral neuropathy. Ann Neurol 2001; 49: 214–22.

Arriagada R, Le Chevalier T, Pignon JP, Rivière A, Monnet I, Chomy P, et al. Initial chemotherapeutic doses and survival in patients with limited small-cell lung cancer. N Engl J Med 1993; 329: 1848–52.

Benyahia B, Liblau R, Merle-Béral H, Tourani JM, Dalmau J, Delattre JY. Cell-mediated autoimmunity in paraneoplastic neurological syndromes with anti-Hu antibodies. Ann Neurol 1999; 45: 162–7.

Byrne T, Mason WP, Posner JB, Dalmau J. Spontaneous neurological improvement in anti-Hu associated encephalomyelitis. J Neurol Neurosurg Psychiatry 1997; 62: 276–8.

Carpentier AF, Rosenfeld MR, Delattre JY, Whalen RG, Posner JB, Dalmau J. DNA vaccination with HuD inhibits growth of a neuroblastoma in mice. Clin Cancer Res 1998; 4: 2819–24.

Chartrand-Lefebvre C, Howarth N, Grenier P, Keime F, Orcel B, Beigelman C. Association of small cell lung cancer and the anti-Hu paraneoplastic syndrome: radiographic and CT findings. Am J Roentgenol 1998; 170: 1513–7.

Cox DR. Regression models and life-tables. J R Stat Soc [B] 1972; 34: 187–220.

Dalmau J, Furneaux HM, Gralla RJ, Kris MG, Posner JB. Detection of the anti-Hu antibody in the serum of patients with small cell lung cancer. A quantitative western blot analysis. Ann Neurol 1990; 27: 544–52.

Dalmau J, Graus F, Rosenblum MK, Posner JB. Anti-Hu-associated paraneoplastic encephalomyelitis/sensory neuronopathy. A clinical study of 71 patients. Medicine (Baltimore) 1992a; 71: 59–72.

Dalmau J, Furneaux HM, Cordon-Cardo C, Posner JB. The expression of the Hu (paraneoplastic encephalomyelitis/sensory neuronopathy) antigen in human normal and tumor tissues. Am J Pathol 1992b; 141: 881–6.

Darnell RB, DeAngelis LM. Regression of small-cell lung carcinoma in patients with paraneoplastic neuronal antibodies. Lancet 1993; 341: 21–2.

Das A, Hochberg FH, McNelis S. A review of the therapy of paraneoplastic neurologic syndromes. [Review]. J Neurooncol 1999; 41: 181–94.

de Toffol B, Uchuya M, Michalak S, Corcia P, Hommet C, Autret A. Encéphalomyélonévrite paranéoplasique avec anticorps anti-Hu et cancer du rectum. Rev Neurol (Paris) 1997; 153: 135–7.

Denny-Brown D. Primary sensory neuropathy with muscular changes associated with carcinoma. J Neurol Neurosurg Psychiatry 1948; 11: 73–87.

Eggers C, Hagel C, Pfeiffer G. Anti-Hu-associated paraneoplastic sensory neuropathy with peripheral nerve demyelination and microvasculitis. J Neurol Sci 1998; 155: 178–81.

Furneaux HM, Rosenblum MK, Dalmau J, Wong E, Woodruff P, Graus F, et al. Selective expression of Purkinje-cell antigens in tumor tissue from patients with paraneoplastic cerebellar degeneration. N Engl J Med 1990; 322: 1844–51.

Giometto B, Scaravilli T, Nicolao P, An SF, Groves M, Tavolato B, et al. Detection of paraneoplastic anti-neuronal autoantibodies on paraffin-embedded tissues. Acta Neuropathol (Berl) 1996; 92: 435–40.

Graus F, Cordon-Cardo C, Posner JB. Neuronal antinuclear antibody in sensory neuronopathy from lung cancer. Neurology 1985; 35: 538–43.

Graus F, Elkon KB, Cordon-Cardo C, Posner JB. Sensory neuronopathy and small cell lung cancer. Antineuronal antibody that also reacts with the tumor. Am J Med 1986; 80: 45–52.

Graus F, Elkon KB, Lloberes P, Ribalta T, Torres A, Ussetti P, et al. Neuronal aninuclear antibody (anti-Hu) in paraneoplastic encephalomyelitis simulating acute polyneuritis. Acta Neurol Scand 1987; 75: 249–52.

Graus F, Vega F, Delattre JY, Bonaventura I, Reñé R, Arbaiza D, et al. Plasmapheresis and antineoplastic treatment in CNS paraneoplastic syndromes with antineuronal auto-antibodies. Neurology 1992; 42: 536–40.

Graus F, Bonaventura I, Uchuya M, Vall-Solé J, Reñé R, Leger JM, et al. Indolent anti-Hu-associated paraneoplastic sensory neuropathy. Neurology 1994; 44: 2258–61.

Graus F, Dalmau J, Reñé R, Torà M, Malats N, Verschuuren JJ, et al. Anti-Hu antibodies in patients with small-cell lung cancer: association with complete response to therapy and improved survival. J Clin Oncol 1997; 15: 2866–72.

Gultekin SH, Dalmau J, Graus Y, Posner JB, Rosenblum MK. Anti-Hu immunolabeling as an index of neuronal differentiation in human brain tumors. A study of 112 central neuroepithelial neoplasms. Am J Surg Pathol 1998; 22: 195–200.

Henson RA, Urich H. Encephalomyelitis with carcinoma. In: Henson RA, Urich H, editors. Cancer and the nervous system. Oxford: Blackwell Scientific; 1982. p. 314–45.

Henson RA, Hoffman HL, Urich H. Encephalomyelitis with carcinoma. Brain 1965; 88: 449–64.

Horwich MS, Cho L, Porro RS, Posner JB. Subacute sensory neuropathy: a remote effect of carcinoma. Ann Neurol 1977; 2: 7–19.

Hosmer DW, Lemeshow S. Applied logistic regression. New York: Wiley; 1989.

Keime-Guibert F, Graus F, Broët P, Reñé R, Molinuevo JL, Ascaso C, et al. Clinical outcome of patients with anti-Hu-associated encephalomyelitis after treatment of the tumor. Neurology 1999; 53: 1719–23.

Keime-Guibert F, Graus F, Fleury A, Reñé R, Honnorat J, Broët P, et al. Treatment of paraneoplastic neurological syndromes with antineuronal antibodies (anti-Hu, anti-Yo) with a combination of immunoglobulins, cyclophosphamide, and methylprednisolone. J Neurol Neurosurg Psychiatry 2000; 68: 479–82.

King PH, Dropcho EJ. Expression of Hel-N1 and Hel-N2 in small-cell lung carcinoma. Ann Neurol 1996; 39: 679–81.

Lucchinetti CF, Kimmel DW, Lennon VA. Paraneoplastic and oncologic profiles of patients seropositive for type 1 antineuronal nuclear auto-antibodies. Neurology 1998; 50: 652–7.

Mason WP, Graus F, Lang B, Honnorat J, Delattre JY, Valldeoriola F, et al. Small-cell lung cancer, paraneoplastic cerebellar degeneration and the Lambert-Eaton myasthenic syndrome. Brain 1997; 120: 1279–300.

Molinuevo JL, Graus F, Serrano C, Reñé R, Guerrero A, Illa I. Utility of anti-Hu antibodies in the diagnosis of paraneoplastic sensory neuropathy. Ann Neurol 1998; 44: 976–80.

Oh SJ, Slaughter R, Harrell L. Paraneoplastic vasculitic neuropathy: a treatable neuropathy. [Review]. Muscle Nerve 1991; 14: 152–6.

Oh SJ, Dropcho EJ, Claussen GC. Anti-Hu-associated paraneoplastic sensory neuropathy responding to early aggressive immunotherapy: report of two cases and review of literature. [Review]. Muscle Nerve 1997; 20: 1576–82.

O'Neill JH, Murray NM, Newsom-Davis J. The Lambert-Eaton myasthenic syndrome. Brain 1988; 111: 577–96.

Pieterman RM, van Putten JW, Meuzelaar JJ, Mooyaart EL, Vaalburg W, Koeter GH, et al. Preoperative staging of non-small-cell lung cancer with positron-emission tomography. N Engl J Med 2000; 343: 254–61.

Posner JB, Dalmau J. Paraneoplastic syndromes. [Review]. Curr Opin Immunol 1997; 9: 723–9.

Shavit YB, Graus F, Probst A, Reñé R, Steck AJ. Epilepsia partialis continua: a new manifestation of anti-Hu-associated paraneoplastic encephalomyelitis. Ann Neurol 1999; 45: 255–8.

Sillevis Smitt PA, Manley GT, Posner JB. Immunization with the paraneoplastic encephalomyelitis antigen HuD does not cause neurologic disease in mice. Neurology 1995; 45: 1873–8.

Spiegelman D, Maurer LH, Ware JH, Perry MC, Chahinian AP, Comis R, et al. Prognostic factors in small-cell carcinoma of the lung: an analysis of 1,521 patients. J Clin Oncol 1989; 7: 344–54.

Szabo A, Dalmau J, Manley G, Rosenfeld M, Wong E, Henson J, et al. HuD, a paraneoplastic encephalomyelitis antigen, contains RNA-binding domains and is homologous to Elav and sex-lethal. Cell 1991; 67: 325–33.

Tim RW, Massey JM, Sanders DB. Lambert-Eaton myasthenic syndrome: electrodiagnostic findings and response to treatment. Neurology 2000; 54: 2176–8.

Turrisi, AT 3rd, Kim K, Blum R, Sause WT, Livingston RB, Komaki R, et al. Twice-daily compared with once-daily thoracic radiotherapy in limited small-cell lung cancer treated concurrently with cisplatin and etoposide. N Engl J Med 1999; 340: 265–71.

Uchuya M, Graus F, Vega F, Reñé R, Delattre JY. Intravenous immunoglobulin treatment in paraneoplastic neurological syndromes with antineuronal autoantibodies. J Neurol Neurosurg Psychiatry 1996; 60: 388–92.

Younger DS, Dalmau J, Inghirami G, Sherman WH, Hays AP. Anti-Hu-associated peripheral nerve and muscle microvasculitis. Neurology 1994; 44: 181–3.

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