Paraneoplastic Neurological Syndromes

Clinical And Serological Studies

Setareh Shamsili

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Promotor:	Prof.dr. P.A.E. Sillevis Smitt
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CHAPTER 1

Introduction

1.1 GENERAL INTRODUCTION

Paraneoplastic Neurological Syndromes

The term "paraneoplastic syndromes" refers to symptoms or signs resulting from damage to organs or tissues that are remote from the site of a malignant neoplasm or its metastases. Paraneoplastic syndromes can affect most organs and tissues including the nervous system. Since the first time that the term "paraneoplastic" was used by Guichard and Vignon in 1949[1] reporting a case with polyradiculopathy and cancer of the uterus, the clinical presentation, associated tumors, the pathogenesis, diagnosis and management of paraneoplastic syndromes including the paraneoplastic neurological syndromes (PNS), has been continuously refined. However, the general concept described by Brain and Norris in 1965 for definition of PNS as a remote effect of cancer still applies[2] . PNS are remote effects of cancer, i.e. not caused by invasion of the tumor or its metastases nor by any adverse event of cancer treatment and not by infection, metabolic disturbances or cerebrovascular complications. The true prevalence of PNS is not yet established and varies between 0.01 - 1 percent of cancer patients. However it is noteworthy to mention much higher frequencies of some syndromes including Lambert-Eaton myasthenic syndrome (LEMS) in 3% of patients with small-cell lung cancer (SCLC)[3], myasthenia gravis in 15% of patients diagnosed with thymoma[4] and a severe predominantly motor neuropathy in about 50% of patients with the osteosclerotic form of plasmacytoma[5].

Despite the rarity of PNS, the importance of these syndromes is that the neurological presentation often antedates the clinical presentation of an underlying cancer with a few months to several years. The most common cancers associated with PNS are breast, ovarian and lung cancer but many other cancers can produce paraneoplastic symptoms as well. The diagnosis of a neurological syndrome as paraneoplastic has been facilitated by the discovery of paraneoplastic antineuronal autoantibodies. These onconeural antibodies are found to be directed against antigens expressed by both the tumor and the nervous system. This finding accompanied by the observation of intrathecal synthesis of these antibodies subsequently resulted in the hypothesis that at least some of these disorders are immune –mediated. However only about 50-60% of patients with PNS harbor paraneoplastic antibodies. Except for very few cases (e.g. anti-VGCC in LEMS and anti-mGluR1 in paraneoplastic cerebellar degeneration) in which paraneoplastic antibodies are proven to have a direct role in the pathogenesis of a PNS, most onconeural antibodies currently might serve only as biomarkers. Each onconeural antibody is associated with specific clinical syndromes and tumor types (Chapter 2 of this thesis, Table 39-2). Therefore detection of an onconeural antibody in the serum of a patient helps diagnose a syndrome as paraneoplastic and also narrows down the search towards the underlying neoplasm when at the time of neurologic presentation the tumor might still be occult.

Even though there is a proven correlation between antibody type, neurological syndrome and tumor type, various levels of clinical complexities exist. These include the association of several clinical syndromes with the same onconeural antibody and the identification of different types of antibodies in the same patient in about 30% of cases. In 2004, the first guidlines on diagnosis of PNS were published by an international panel of PNS experts. According to these guidelines, a neurological

syndrome can be classified as definitely or possibly paraneoplastic, based on the presence or absence of cancer, the presence of a classical syndrome, presence of partially or well characterized onconeural antibodies and achieving improvement after cancer therapy[6]. The term "classical syndrome" applies to those neurological syndromes that often associate with cancer. The diagnosis of a classical syndrome should prompt the investigation of an occult tumor regardless of the antibody status. Currently eight syndromes are accurately described as classic which are highly suggestive of PNS[7].These syndromes include: encephalomyelitis, limbic encephalitis, subacute cerebellar degeneration, opsoclonus-myoclonus, subacute sensory neuronopathy, chronic gastrointestinal pseudo-obstruction, Lambert Eaton myasthenic syndrome and dermatomyositis.

Classical syndromes involving the central nervous system

Encephalomyelitis

Paraneoplastic encephalomyelitis (PEM) is defined by disseminated neuronal loss and may present with various clinical syndromes including but not limited to limbic encephalitis, brain stem encephalitis, cerebellar degeneration, myelitis and neuropathies[8-11]. The most common antineuronal antibodies associated with PEM are anti-Hu (ANNA-1), anti-CV2 (CRMP5) and anti-amphiphysin. However in some patients with PEM, no well defined antineuronal antibody can be detected, suggesting that other, as yet unknown, antigens may trigger the extensive neuronal damage. The majority of cases with a cancer are reported to be a lung tumor (80%) with SCLC to comprise about 60% of them[12-15].

Limbic encephalitis

The clinical characteristics of limbic encephalitis (LE) include an acute or subacute course (days up to 12 weeks), seizures, short term memory loss, confusion, and psychiatric symptoms. Since the first description of this syndrome in 1960[16-17]until recently, LE was considered to be almost always paraneoplastic with poor outcome and in 50-60% of cases to be accompanied by onconeural antibodies (anti-Hu, anti-Ma2, anti-CV2) with most common associated tumors recognized as SCLC or testicular cancer.

More recently, antibodies directed against voltage-gated potassium channels (anti-VGKC) have been associated with a non-paraneoplastic variant of LE, Morvan syndrome, isolated epilepsy and neuromyotonia [18-19].The anti-VGKC associated LE is more prevalent in men and presents with classical symptoms of LE in addition to REM sleep behavior disorder and frequently hyponatriemia. Serum anti-VGKC titers are high without intrathecal synthesis. A recent study showed that 10% of patients had neurological symptoms different from those of LE and 14% was diagnosed with Creutzfeld-Jakob disease like syndrome. In addition, 31% had an underlying tumor, usually SCLC or thymoma [20].

Onconeural antibodies were until recently considered to be present in 50-60% of LE patients with cancer. Many cases that were considered 'sero-negative' may in fact harbor antibodies directed against cell surface antigens [21]. Two of these antibodies have recently been identified, reacting with the glutamate receptor1(GluR1) and GluR2 subunits of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic

acid receptor (AMPAR) [22] or the B subunit of the gamma-aminobutyric acid ($GABA_B$) receptor respectively [23]. A tumor (usually SCLC) is detected in 47% of anti-$GABA_B$ receptor associated LE and in 64% of cases associated with anti-AMPAR (usually SCLC followed by thymoma and breast cancer). Clinically anti-AMPAR associated LE occurs almost exclusively in women and has a tendency to relapse while anti-GABAB receptor LE presents early on with prominent seizures and is associated with the presence of other autoantibodies, mainly anti-GAD. A severe but treatment-responsive form of encephalitis has been associated with antibodies against the NR1 subunit of the N-methyl-D-aspartate receptor (anti-NMDAR) [24-25], Anti-NMDAR encephalitis occurs most frequently in young women and children and presents with psychiatric symptoms followed by seizures, decline of consciousness, aphasia and abnormal movements [24-26]. Patients frequently suffer autonomic failure and hypoventilation requiring admission to the intensive care unit. An underlying teratoma is found in 56% of women older than 18 years and in only 9% of girls under the age of 9 [26]. In boys with NMDAR encephalitis, generally no underlying tumor was found [26], while in older men SCLC and immature teratoma were described [24].
Despite the severity of symptoms, up to 75% of patients recover fully with mild deficits. Patients who are treated promptly with removal of the tumor and with immunosuppression recover better than those who are not treated or only receive immunotherapy, either because no tumor is present or because it was missed initially [26].

Subacute cerebellar degeneration

Paraneoplastic cerebellar degeneration (PCD) with its selective loss of Purkinje neurons is one of the most common PNS. Categorizing a cerebellar dysfunction as a classical paraneoplastic syndrome depends on a subacute onset with pancerebellar involvement. In the acute phase, MRI may show enhancement followed by atrophy in the chronic phase [27]. The most common onconeural antibody associated with PCD is anti-Yo, which is usually associated with a gynecological or breast malignancy. Detection of anti-Tr should direct the search towards Hodgkin's lymphoma. In addition, although less specific for PCD, anti-CV2 and anti-Hu are common[28-29]. About 50% of PCD are not associated with well characterized antibodies.

Opsoclonus-myoclonus

Paraneoplastic opsoclonus-myoclonus (POM) is characterized by acute or subacute onset of abnormal eye movements in any direction and myoclonic jerks with or without cerebellar signs. POM is observed in about 50% of pediatric patients with neuroblastoma[30]. In adults, the associated tumors are lung and breast cancer or gynecological cancer such as uterus or ovary. However, individual cases of a variety of other tumors are also reported[31-32]. The most commonly associated onconeural antibodies are anti-Ri (mostly in female patients) and anti-Hu although anti-amphiphysin and anti-Ma have also been reported.

Classical syndromes involving the peripheral nervous system, neuromuscular junction or muscle

Subacute sensory neuronopathy

Paraneoplastic sensory neuronopathy (PSN) is not always an isolated syndrome and paraneoplasia is only one of the causes of this syndrome. In the classical form, symptoms at onset are asymmetrical, arms are always involved and electrophysiological studies show marked involvement of the sensory fibers with absent sensory nerve action potentials in at least one of the nerves studied[6, 33] .The most common associated tumor and antibody with PSN are SCLC and anti-Hu respectively[11, 34].

Gastrointestinal pseudo-obstruction

Chronic gastrointestinal pseudo-obstruction is caused by gastroparesis resulting from damage to the neurons of the enteric plexus. Symptoms consist of severe constipation, dysphagia, and vomiting. The most commonly associated tumor is SCLC and the most common antineuronal antibodies reported are anti-Hu and anti-CV2[7, 35].

Lambert–Eaton myasthenic syndrome

Lambert–Eaton myasthenic syndrome (LEMS) is a disorder of the neuromuscular junction. When paraneoplastic (60%), SCLC is the primary associated cancer. Almost all patients with LEMS have autoantibodies against voltage gated calcium channel antibodies (anti-VGCC) in their serum, irrespective of a paraneoplastic or autoimmune etiology[36-37]. Differentiation of paraneoplastic from non-paraneoplastic is facilitated by smoking history, HLA-typing and detection of SOX and ZIC autoantibodies [15, 38-39].

Antineuronal antibodies

The currently available diagnostic guidelines for PNS distinguish between well characterized onconeural antibodies (against Hu, Yo, Ri, amphiphysin, CV2 and Ma2) and partially characterized antibodies (against Tr, ANNA3, PCA2, Zic4, and mGluR1[6].The third category consists of antineuronal antibodies that may be associated with both paraneoplastic and non-paraneoplastic neurological syndromes (e.g. anti-VGCC).

Over the past few years a number of new antineuronal antibodies have been identified that are directed against neuronal cell surface receptors, expressed in the central nervous system [21]. These autoantibodies are associated with distinct forms of autoimmune encephalitis that may or may not be paraneoplastic, often presenting as typical LE. As opposed to the onconeural antibodies reactive with intracellular antigens, the autoantibodies against NMDAR, mGluR1 and other neuronal cell surface receptors are probably pathogenic [40-41]. In addition, the clinical syndromes associated with these antibodies against neuronal cell surface receptors respond better to immunotherapy.

Based on these findings a new classification of neuronal antibodies associated with syndromes resulting from dysfunction of the central nervous system has been proposed [21]. Group I antibodies react with intracellular antigens while group

II antibodies react with neuronal cell surface antigens (Table 1). Group I is further subdivided in three groups. Group Ia consists of the well characterized onconeural antibodies; group Ib antibodies (Zic and SOX) are cancer-specific while group Ic antibodies identify non-paraneoplastic syndromes. Group IIa antibodies can be associated with both paraneoplastic and with non-tumor associated neurological syndromes while group IIb antibodies are paraneoplastic.

Treatment and prognosis

The prognosis of PNS associated with the well characterized onconeural antibodies is generally poor [11, 28, 42] and depends on the severity of the neurological syndrome, associated antibody and the underlying tumor. Case series, mostly retrospective, suggest that treatment of the tumor, with or without immunotherapy, is an independent predictor of improvement or stabilization of the neurological disfunction[11, 28, 42]. The pleomorphic presentation of PNS still causes profound diagnostic delay, despite the general availability of paraneoplastic antibody testing [43]. Based on the autoimmune hypothesis for the etiology of PNS, various attempts with different types of conventional immunotherapy including administration of corticosteroids, intravenous immunoglobulin, plasmapheresis and immunosupression with cyclophosphamide are tried[44-45]. In addition newer agents such as rituximab have been tried in a limited number of patients[46]. The use of tacrolimus and sirolimus, oral agents approved for immunosupression in organ transplantation, is also suggested[47] but results of clinical trials in PNS are not available. So far, the results of various forms of immunotherapy have been disappointing with some exceptions[48].

It seems that PNS associated with Group II antibodies, directed against neuronal cell surface antigens, are more responsive to treatment and therefore have a better prognosis. The majority of patients (75%) with NMDAR encephalitis recover fully or with mild deficits [25-26] while benefit from immunotherapy was reported in 89% of patients with LE and VGKC antibodies that was marked in 50% [20].

Table 1. Neuronal antibodies associated with paraneoplastic and nonparaneoplastic neurological syndromes of the central nervous system

Antibody	Predominant tumor (%)[a]	CNS syndrome	Comments
Group I: Antibodies against intracellular antigens			
Group Ia: PNS-related onconeural antigen (well characterized)			
Hu (ANNA-1)	SCLC (98)	Encephalomyelitis; Paraneoplastic cerebellar degeneration; Limbic encephalitis; Brainstem encephalitis	Frequency in cancer without PNS: 16%
CV2 (CRMP5)	SCLC, thymoma (96)	Encephalomyelitis; Chorea; PCD; Limbic encephalitis	Frequency in cancer without PNS: 9%
Amphiphysin	Breast, SCLC (95)	Stiff-person syndrome; Myelopathy and myoclonus; Encephalomyelitis	Frequency in cancer without PNS: 1%
Ri (ANNA-2)	Breast, SCLC (97)	Brainstem encephalitis; Opsoclonus myoclonus	Frequency in cancer without PNS: 4%
Yo (PCA-1)	Ovary, breast (98)	PCD	Frequency in cancer without PNS: 1%
Ma2	Testicular (96)	Limbic encephalitis; Brainstem encephalitis	Frequency in cancer without PNS: 0%
Group Ib: Cancer-related onconeural antibodies			
ZIC	SCLC	PCD	Frequency in cancer without PNS: 15%
SOX	SCLC	LEMS	Frequency in cancer without PNS: 36%
Group Ic: Non-paraneoplastic antibodies associated with CNS syndromes			
GAD-65	NA	SPS; cerebellar ataxia	Frequency in diabetes mellitus: 80%
Adenylate kinase 5	NA	Limbic encephalitis	
Homer 3	NA	Cerebellar ataxia	
Group II: Antibodies against neuronal surface antigens			
Group IIa: Markers of the CNS syndrome			
VGKC	SCLC, thymoma (31)	Limbic encephalitis; Morphan syndrome; Rapidly progressive dementia (CJD like)	Male predominance, REM sleep behavior disorder, hyponatremia (70%)
NMDA receptor	Ovarian teratoma (56%)	Encephalitis with initial psychiatric presentation, catatonia, dystonia, aphasia and hypoventilation	Female predominance; MRI normal in 45%, in patients >18 years tumor frequency higher
AMPA receptor	SCLC, breast, thymoma (70)	Limbic encephalitis; Atypical psychosis	Female predominance; frequent relapses (60%)
GABAB receptor	SCLC (47)	Limbic encephalitis	Seizures in 86%, concurrent GAD ab
Glycine receptor	Lung cancer	Progressive encephalomyelitis with rigidity, SPS	Only one patient published
Group IIb: Markers of a CNS paraneoplastic syndrome			
mGluR1	Hodgkin's disease	PCD	Only two patients
VGCC	SCLC	PCD	With or without LEMS

[a] Percentage denotes the percentage of patients with any underlying tumor

PNS, paraneoplastic neurological syndrome; CNS, central nervous system; ANNA, anti-neuronal nuclear antibody; SCLC, small cell lung cancer; PCD, paraneoplastic cerebellar degeneration; SPS, stiff-person syndrome; PCA, Purkinje cell antibody; LEMS, Lambert-Eaton myasthenic syndrome; VGKC, voltage-gated potassium channels; NMDA, N-methyl-D-aspartate; AMPA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; GABA, gamma-aminobutyric acid; VGCC, voltage-gated calcium channels.

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1.2 AIM AND SCOPE OF THIS THESIS

The identification of onconeural antibodies has improved the diagnosis of paraneoplastic neurological syndromes (PNS) and subsequent early detection and treatment of an occult malignancy. However, still there is a significant unmet need for effective treatments to improve or stabilize the neurological outcome of the patient. The aim of this thesis was to improve the diagnosis and treatment of PNS. To reach this aim, we expanded the description of antineuronal antibodies and the associated paraneoplastic neurological syndromes and tumors; identified a novel onconeural antibody and performed a prospective clinical trial to study efficacy and safety of rituximab, a monoclonal antibody, in PNS.

Chapter 2 provides a detailed review of PNS including classification, definition, diagnosis, underlying malignancies, treatment, prognosis and description of related onconeuronal auto-antibodies. Some of the more recent developments have been summarized in Chapter 1.

Chapter 3 is a retrospective investigation of antineuronal antibodies associated with paraneoplastic cerebellar degeneration (PCD). By screening >5000 blood samples over a 12 years period, we identified 50 patients with high titer onconeural antibodies and PCD. We examined the relative frequency of the antineuronal antibodies associated with PCD and compared the neurological symptoms and signs, associated tumors, disability and survival between groups of PCD with different antibodies. Also, we attempted to identify patient-, tumor-, and treatment-related characteristics associated with functional outcome and survival.

Hodgkin's disease (HD) is the third most common cause of PCD after lung and gynecological cancer. Preliminary studies suggested a strong correlation between HD, PCD and anti-Tr antibodies. **Chapter 4** describes our extended clinical–immunologic analysis of 28 patients with anti-Tr antibodies. We identified clinical characteristics and associated tumors, determined the course of anti-Tr titers over time and identified the IgG subclasses of anti-Tr. In addition, expression of the Tr antigen was examined in 15 HD tumor samples and one autopsied patient's brain was examined for cell loss and inflammation.

Not all patients with paraneoplastic encephalomyelitis (PEM) have detectable well or partially characterized onconeuronal antibodies, suggesting that other, as yet unknown, antigens may trigger the extensive neuronal damage. In **Chapter 5,** we describe the detection of a new antibody in a patient with PEM and SCLC with immunoreactivity against the axon initial segment (AIS), detected by immunohistochemical methods. We started the characterization of the antigen by excluding other previously identified antigens and then proceeded by screening a rat hippocampal cDNA library attempting to isolate the target antigen.

Early treatment of the underlying tumor is the cornerstone of treatment of PSN. However, despite aggressive antitumor management, the neurological and

functional outcome has remained grim in many patients. We hypothesized that elimination of circulating B lymphocytes in patients with newly diagnosed anti-Hu and anti-Yo associated PNS would result in the prevention of the development of antibody secreting cells, in a reduction of autoantibody titers and in clinical improvement or stabilization of disease. In addition, elimination of circulating B cells may diminish ongoing paraneoplastic antigen presentation. CD20 is a surface membrane antigen expressed mainly by B cell precursors and mature B cells and appears to play an important functional role in B cell activation, proliferation and differentiation. **Chapter 6** describes our prospective clinical study in 9 PNS patients, treated with rituximab a chimeric anti-CD20 monoclonal antibody that is approved for the treatment of CD20+ B-cell non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia (CLL).

Finally, the work presented in this thesis is summarized and discussed in **Chapter 7** and a Dutch translation of its contents is provided in **chapter 8**.

CHAPTER 2

Paraneoplastic Neurologic Syndromes

Paraneoplasia

Setareh Shams'ili / Peter Sillevis Smitt

Principles of Neuro-Oncology, 2005, p (649-677)

By definition, paraneoplastic neurologic syndromes (PNS) are remote effects of cancer that are not caused by invasion of the tumor or its metastases nor by infection, cerebrovascular complications, surgery, or other forms of tumor treatment.[1] Some clinical syndromes, such as subacute cerebellar degeneration,[2] subacute sensory neuronopathy,[3] opsoclonus/myoclonus,[4] and limbic encephalitis,[5] are relatively often paraneoplastic and are preferentially associated with certain tumors (Tables 30-1 and 30-2). In these cases a careful and directed search for an underlying neoplasm should be conducted. When more common neurologic syndromes such as mixed somatic polyneuropathy[6] and chronic inflammatory demyelinating polyneuropathy (CIDP)[7] occur in close temporal relation with a relatively frequent event as cancer, a chance association should be considered. A paraneoplastic etiology depends in these instances on the relative frequencies of tumor and non-tumor cases. In an individual patient, a paraneoplastic etiology is strongly suggested when improvement of the neurologic syndrome coincides with treatment of the tumor. The discovery of paraneoplastic antineuronal autoantibodies has facilitated the diagnosis of a neurologic syndrome as paraneoplastic. The paraneoplastic or onconeural antibodies are associated with specific clinical syndromes and tumor types. The high specificity of these antibodies helps diagnose a syndrome as paraneoplastic and directs the search toward an underlying neoplasm. The discovery of paraneoplastic autoantibodies has also led to the autoimmune hypothesis of paraneoplastic disorders. This hypothesis postulates that an immune response directed against neuronal antigens expressed by the tumor cross-reacts with the same or similar antigens in the nervous system.[8] Intrathecal synthesis of the paraneoplastic antibodies and the finding that the antibodies are directed against antigens expressed in affected parts of the nervous system support the autoimmune hypothesis of paraneoplasia.[9] Despite the presumed autoimmune etiology of the paraneoplastic neurologic syndromes, the results of various forms of immunotherapy with some exceptions have been generally disappointing. Detection and subsequent treatment of the underlying tumor appears to offer the best chance of preventing neurologic deterioration.

► **TABLE 30-1.** PARANEOPLASTIC NEUROLOGIC SYNDROMES*

Central nervous system
Subacute cerebellar ataxia
Limbic encephalitis
Encephalomyelitis
Opsoclonus–myoclonus
Stiff-person syndrome
Chorea
Cancer and melanoma-associated retinopathy
Optic neuritis
Subacute motor neuronopathy/motor neuron disease
Peripheral nervous system
Subacute sensory neuronopathy
Sensorimotor neuropathy
Sensorimotor neuropathy associated with M protein
Subacute autonomic neuropathy
Vasculitis of nerve and muscle
Neuromyotonia
Neuromuscular Junction and Muscle
Lambert-Eaton myasthenic syndrome
Myasthenia gravis
Dermatomyositis/polymyositis
Acute necrotizing myopathy
Paraneoplastic carcinoid myopathy
Cachectic myopathy

*In *italic* are clinical syndromes that are relatively often paraneoplastic. Because these syndromes are preferentially associated with certain tumors, a careful and directed search for an underlying neoplasm should be conducted.

► SPECIFIC CLINICAL PRESENTATIONS

PNS are characterized by the association of specific neurologic syndromes with typical paraneoplastic antibodies and tumor types. The most common associations are summarized in Tables 30-2 and 30-3. Characteristic clinical presentations are discussed below.

Central Nervous System

Subacute Cerebellar Ataxia

Paraneoplastic cerebellar degeneration (PCD) is one of the most common and characteristic paraneoplastic syndromes.[10,11] In a study of 137 consecutive patients with PNS associated with a paraneoplastic antibody, 50 (37%) presented with subacute cerebellar ataxia.[11] Brouwer first described PCD in 1919,[12] but the association with cancer was not recognized until 1938.[13] PCD usually starts with slight incoordination of walking, but evolves rapidly over weeks to a few months with progressive appendicular, truncal, and gait ataxia, dysarthria, and often nystagmus with oscillopsia. The disease reaches its peak within months and then stabilizes. By this time, most patients are severely debilitated. They are generally unable to walk without support and may be unable to sit unsupported. Handwriting is often illegible and feeding oneself difficult. The neurologic signs are always bilateral but may be asymmetric. Diplopia is common at presentation, although the investigator usually cannot detect abnormalities of ocular movement. Symptoms and signs are limited to the cerebellum and cerebellar pathways, but other mild neurologic abnormalities may be found on careful examination. These include hearing loss, dysphagia, pyramidal and extrapyramidal tract signs, mental status change, peripheral neuropathy, and Lambert-Eaton myasthenic syndrome (LEMS).[14–17] Magnetic resonance imaging (MRI) and computerized tomography (CT) scan are initially normal but often reveal cerebellar atrophy later in the course of the disease. Cerebral spinal fluid (CSF) examination, carried out to exclude leptomeningeal metastases, shows mild lymphocytic pleocytosis with elevation of protein and immunoglobulin (Ig) G levels in the first weeks to months. Oligoclonal bands may be present.

Underlying Tumor

PCD can be associated with any cancer but the most common tumors are lung cancer (usually small cell lung cancer (SCLC)), ovarian cancer, and lymphomas (particularly Hodgkin disease). In 60% to 70% of the patients, the neurologic symptoms precede the diagnosis of the cancer by a few months to 2 to 3 years and lead to its detection.[11,17,18]

Diagnostic Evaluation

PCD is a rare disorder in cancer patients. Conversely, 50% of patients presenting with acute or subacute nonfamilial ataxia are estimated to have an underlying malignancy.[10] The diagnosis of PCD is established by demonstration of specific antineuronal antibodies. The type of antibody directs the search for an underlying neoplasm (see Table 30-2).

Antineuronal Antibodies

PCD can be associated with various antineuronal autoantibodies. Anti-Yo, anti-Tr, anti-voltage-gated calcium channel (VGCC), anti-Ri, and anti-mGluR1 are associated with relatively "pure" cerebellar syndromes (Fig. 30-1).[11,19,20] Approximately 50% of patients presenting with PCD and an underlying SCLC have high titer anti-Hu antibodies.[14] In these patients, PCD is part of the paraneoplastic encephalomyelitis (PEM)/sensory neuronopathy (SN) syndrome, and more widespread neurologic symptoms and signs are usually found. In patients with SCLC and PCD without additional neurologic manifestations, anti-VGCC antibodies are more common (41%) than anti-Hu antibodies (16%).[20] Approximately half of the patients with PCD and anti-VGCC antibodies have additional clinical or electrophysiologic signs of LEMS.[14,20] Anti-Ri (ANNA-2) antibodies combine PCD and paraneoplastic opsoclonus

► **TABLE 30-2.** NEUROLOGIC SYNDROMES AND THE ROLE OF SPECIFIC AUTOANTIBODIES IN ESTABLISHING A PARANEOPLASTIC ETIOLOGY AND DIRECTING THE SEARCH FOR AN UNDERLYING NEOPLASM

Syndrome	Antibody	Paraneoplastic Antibody	Cancer (Most Common)
Central nervous system			
Subacute cerebellar degeneration		Anti-Yo	Ovary, breast
		Anti-Hu	SCLC
		Anti-Tr	Hodgkin disease
		Anti-Ri	Breast, gynecologic
		Anti-VGCC	SCLC
		Anti-mGluR1	Hodgkin disease
		PCA-2	SCLC
		ANNA-3	SCLC
		Anti-Ma	Miscellaneous
		Anti-Zic4	SCLC
Limbic encephalitis		Anti-Hu	SCLC
		Anti-Ta/Ma2	Testicular seminoma
		Anti-Ma	Lung cancer and other cancers
		ANNA-3	Lung cancer
		Anti-CRMP5/CV2	SCLC, thymoma
Encephalomyelitis		Anti-Hu	SCLC
		Anti-amphiphysin	SCLC, breast
		Anti-CRMP5/CV2	SCLC
		ANNA-3	Lung cancer
		PCA-2	SCLC
Opsoclonus–myoclonus (infant)		Anti-Hu	Neuroblastoma
Opsoclonus–myoclonus (adult)		Anti-Hu	SCLC
		Anti-Ri	Breast, gynecologic, bladder, SCLC
Stiff-person syndrome	Anti-GAD	Anti-amphiphysin	Breast, SCLC
Extrapyramidal syndrome		Anti-CRMP5/CV2	SCLC, lymphoma, renal cancer
		Anti-Hu	SCLC
Cancer-associated retinopathy		Anti-recoverin	SCLC
Cancer-associated melanoma		Antibodies against retinal bipolar cells	Melanoma
Optic neuritis		Anti-CRMP5/CV2	SCLC, thymoma
		Anti-Hu	SCLC
Motor neuronopathy (MND)		Anti-Hu	SCLC
Peripheral nervous system			
Subacute sensory neuronopathy		Anti-Hu	SCLC
		Anti-amphiphysin	SCLC
		Anti-CRMP5/CV2	SCLC, thymoma
		ANNA-3	Lung cancer
		PCA-2	SCLC
Sensorimotor neuropathy		Anti-Hu	SCLC
		Anti-CRMP5/CV2	SCLC, thymoma
Sensorimotor neuropathy-associated M protein		IgG or IgM M proteins	Multiple myeloma, osteosclerotic myeloma, B-cell NHL, B-cell CLL
		Anti-MAG IgM	Waldenström macroglobulinemia
Autonomic neuropathy	Anti-nAchR	Anti-Hu	SCLC
Neuromyotonia	Anti-VGKC	NA	Thymoma, lung cancer
Neuromuscular junction and Muscle			
Lambert-Eaton myasthenic syndrome	Anti-VGCC	NA	SCLC
Myasthenia gravis	Anti-AchR Anti-MuSK	Anti-titin	Thymoma
Dermatomyositis, polymyositis	Anti-Jo-1 Anti-Mi-2 Anti-PM-Sc1	NA	Various

ANNA = antineuronal nuclear antibody; CLL = chronic lymphocytic leukemia; GAD = glutamic acid decarboxylase; MAG = myelin-associated glycoprotein; mGluR1 = metabotropic glutamate receptor type 1; MuSK = muscle specific kinase; NA = not applicable; nAChR = nicotinic acetylcholine receptor; NHL = non-Hodgkin lymphoma; PCA = Purkinje cytoplasmic antibody; SCLC = small cell lung cancer; VGCC = voltage-gated calcium channels; VGKC = voltage-gated potassium channel.

► **TABLE 30-3.** PARANEOPLASTIC ANTIBODIES

Antibody	Clinical Syndromes	Associated Cancer	Neuronal Immunoreactivity	Protein Antigens	Genes	Protein Function	Reference
Anti-Hu (ANNA-1)	Paraneoplastic encephalomyelitis, paraneoplastic limbic encephalitis, paraneoplastic sensory neuronopathy, paraneoplastic cerebellar degeneration, autonomic neuropathy	SCLC, neuroblastoma, prostate cancer	Nucleus more than cytoplasm of all neurons, nucleolar sparing	35–40 kDa	*HuD, HuC, Hel-N1*	RNA binding	36, 173
Anti-Yo (PCA-1)	Paraneoplastic cerebellar degeneration	Ovarian, breast, and lung cancer	Cytoplasm of Purkinje cells and large brainstem neurons	34 and 62 kDa	*cdr34, cdr62*	DNA binding	174, 175
Anti-Ri (ANNA-2)	Cerebellar ataxia with or without opsoclonus and myoclonus	Breast, gynecologic, and bladder cancer and SCLC	Nucleus more than cytoplasm of all central neurons, nucleolar sparing	55 and 80 kDa	*Nova*	RNA binding	176, 177
Anti-Tr (PCA-Tr)	Paraneoplastic cerebellar degeneration	Hodgkin lymphoma	Cytoplasm and dendrites of Purkinje cells	?	Unknown	Unknown	18, 178, 179
Anti-CRMP5 (anti-CV2)	Paraneoplastic encephalomyelitis, paraneoplastic limbic encephalitis, paraneoplastic sensory neuronopathy, sensorimotor neuropathy, chorea, optic neuritis, paraneoplastic cerebellar degeneration, autonomic neuropathy	SCLC, thymoma	Cytoplasm of oligodendrocytes and neurons	60 kDa	*CRMP5* (*POP66*)	Neuronal development	21, 22
Anti-VGCC	Lambert-Eaton myasthenic syndrome, paraneoplastic cerebellar degeneration	SCLC	Presynaptic neuromuscular junction	64 kDa (P/Q type VGCC)	*CACNA1A, MysB*	Transmission at the nerve–muscle synapse	14, 20
Anti-amphiphysin	Stiff-person syndrome, paraneoplastic encephalomyelitis, paraneoplastic sensory neuronopathy, paraneoplastic cerebellar degeneration	Breast cancer, SCLC	Presynaptic nerve terminals	128 kDa	Amphiphysin	Synaptic vesicle endocytosis	39, 57, 60

PCA-2	Paraneoplastic encephalomyelitis, paraneoplastic cerebellar degeneration, motor neuropathy, autonomic neuropathy	SCLC	Cytoplasm of Purkinje cells and other neurons	280 kDa	—	—	23
Anti-retinal	Cancer-associated retinopathy Melanoma-associated retinopathy	SCLC Melanoma	Photoreceptors, ganglion cells Retinal bipolar cells	23, 65, 145, and 205 kDa	Recoverin	Light-dark adaptation by regulating Ca-dependent rhodopsin phosphorylation	77 74
Anti-Ma	Limbic brainstem encephalitis, paraneoplastic cerebellar degeneration	Lung cancer and other cancers	Nuclei and cytoplasm of neurons	40 kDa	*Ma1-3*	Unknown	29
Anti-Ta/Ma2	Limbic brainstem encephalitis	Testicular cancer	Nuclei and cytoplasm of neurons	40 kDa	*Ma2*	Unknown	26, 29
ANNA-3	Paraneoplastic encephalomyelitis, paraneoplastic sensory neuronopathy	Lung cancer	Nuclei of Purkinje cells and other neurons	170 kDa	—	—	40
Anti-mGluR1	Paraneoplastic cerebellar degeneration	Hodgkin lymphoma	Cytoplasm of Purkinje cells and brush cells, climbing fibers	160 kDa	*mGluR1*	Signal transmission by coupling G proteins in the cytoplasm, motor learning	19
Anti-Zic4	Paraneoplastic cerebellar degeneration	SCLC	Nuclei of cerebellar granule cells and other neurons	55, 50, 48, and 35–38 kDa	*Zic4*	Zinc-finger protein, cerebellar development	180
Anti-nAChR	Subacute autonomic neuropathy	SCLC	Postsynaptic, autonomic ganglia	Neuronal ganglionic nicotinic acetylcholine receptor	Neuronal ganglionic nicotinic acetylcholine receptor	Fast synaptic transmission through autonomic ganglia	120, 181

(*Continued*)

► **TABLE 30-3.** PARANEOPLASTIC ANTIBODIES (*CONTINUED*)

Antibody	Clinical Syndromes	Associated Cancer	Neuronal Immunoreactivity	Protein Antigens	Genes	Protein Function	Reference
Anti-VGKC	Limbic encephalitis, peripheral nerve hyperexcitability (neuromyotonia)	Thymoma, SCLC	Peripheral nerve	VGKC	Potassium channels	Controlling neuronal excitability	128, 182
Anti-MAG	Peripheral neuropathy	Waldenström macroglobulinemia	Peripheral nerve	107 kDa	MAG	Glia–axon interactions	183, 184

ANNA = antineuronal nuclear antibody; MAG = myelin-associated glycoprotein; mGluR1 = metabotropic glutamate receptor type 1; nAChR = nicotinic acetylcholine receptor; PCA = Purkinje cytoplasmic antibody; SCLC = small cell lung carcinoma; VGCC = voltage-gated calcium channels; VGKC = voltage-gated potassium channel.

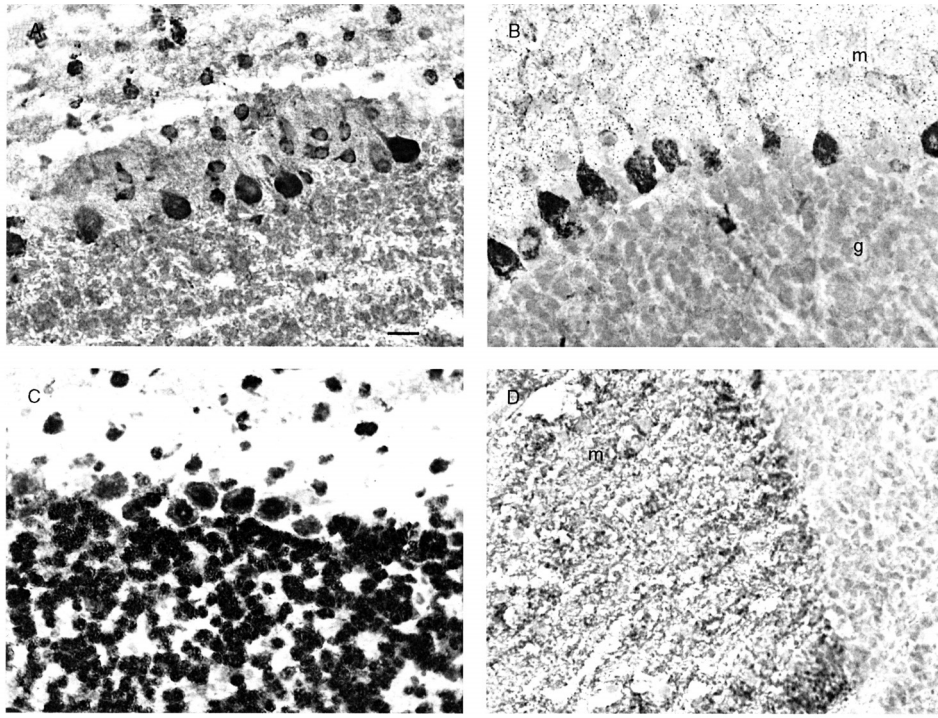


Figure 30-1 (PLATE 60). Paraneoplastic antibodies associated with "pure" PCD. (A) Anti-Yo antibodies label cytoplasm of Purkinje cells. (B) Incubation with anti-Tr antibodies results in strong immunoreactivity of Purkinje cell cytoplasm and dot-like labeling of the molecular layer. (C) Anti-Ri antibodies stain nuclei more than cytoplasm of all central neurons with sparing of the nucleolus. (D) Anti-mGluR1 strongly stains molecular layer and, to a lesser extent, the Purkinje cells. Frozen sections of rat cerebellum were incubated with the patients' sera after acetone fixation. The scale bar represents 20 μm; m = granular layer; g = molecular layer.

and myoclonus (see below). Patients with antibodies directed against amphiphysin and CRMP-5 (anti-CV2) usually do not present with symptoms of PCD, although cerebellar ataxia may become part of the paraneoplastic syndrome later in the course of the disease in 25% of patients.[21,22] The more recently discovered Purkinje cell antibody (PCA-2) is associated with lung cancer and a variety of neurologic syndromes, including PCD.[23]

Differential Diagnosis

The differential diagnosis of PCD includes alcohol intake, vitamin E deficiency, multiple sclerosis, cerebral vasculitis, chronic central nervous system (CNS) infection, systemic autoimmune diseases, celiac disease, metabolic and endocrine encephalopathies, hereditary spinocerebellar degenerations, and primary neurodegenerative disorders. In patients with cancer, intracerebral and leptomeningeal metastases and infections should be excluded.

Treatment and Prognosis

The outcome of PCD is generally poor and no recommended treatment exists. Incidental improvement has been reported either spontaneously, in response to tumor excision, or in association with plasma-exchange, steroids, intravenous immunoglobulin (IVIg), or cyclophosphamide. In a recent report of 34 women with anti-Yo–associated

PCD, the only independent predictor for survival was the type of associated tumor.[17] The median survival was 100 months for patients with breast cancer and 22 months for those with gynecologic cancer. Although PCD leads to the diagnosis of cancer in 60% to 70% of patients, cancer progression is the cause of death in approximately 50% of patients.[11,15–17] The neurologic outcome in anti-Yo–associated PCD is grim: 80% to 94% of patients become bed- or chair-bound, and neurologic improvement rarely occurs.[11,17] The picture is somewhat less gloomy in anti-Ri–, anti-mGluR1–, and anti-Tr–associated PCD. With successful treatment of the tumor and/or immunotherapy, symptoms may disappear.[11,18,19]

Limbic Encephalitis

Paraneoplastic limbic encephalitis (PLE) is a rare disorder characterized by the subacute onset (in days to a few months) of short-term memory loss, seizures, confusion, and psychiatric symptoms, suggesting involvement of the limbic system.[10,24] Selective impairment of recent memory is a hallmark of the disease but may not be evident in patients presenting with severe confusion or multiple seizures.[5] More than half of patients presenting with limbic encephalitis may have PLE.[5] Clinically three groups of patients with PLE can be identified.[5] The first group consists of patients with anti-Hu antibodies and lung cancer (usually SCLC). The PLE is part of PEM, and these patients have involvement of other areas outside the limbic system and brainstem. They are older (median age: 62 years), usually smoke, and are more often female.[5,25] The second group consists of young males with testicular cancer and anti-Ta antibodies.[26] The median age of the second group of patients is 34 years. Symptoms are usually confined to the limbic system, hypothalamus, and brainstem. The third group has no antineuronal antibodies (approximately 40% of patients with PLE).[5,27] In these patients, the symptoms are more often confined to the limbic system, the median age is around 57 years, and the associated tumor is often located in the lung.[5,25] MR and CT scan are abnormal in 65% to 80% of patients.[5,27] Abnormalities consist of increased signal on T_2-weighted and fluid-attenuated inversion recovery (FLAIR) images of one or both medial temporal lobes, hypothalamus, and brainstem (Fig. 30-2). Early in the course of the disease, the MRI may be normal and repeat imaging may be indicated. Coregistration of [^{18}F]fluorodeoxyglucose positron emission tomography (FDG-PET) may further improve the imaging sensitivity.[28] CSF examination is abnormal in 80% of PLE patients, showing transient mild lymphocytic pleocytosis with increased protein, IgG, or oligoclonal bands.[5,27]

Underlying Tumor

SCLC is present in 40% to 55% of patients; testicular cancer is present in approximately 20% of patients.[5,25,27] Other tumors include non-SCLC, breast cancer, Hodgkin disease, and immature teratomas, as well as many other cancers.[5,27]

Diagnostic Evaluation

The diagnosis is often difficult because there are no specific clinical markers and symptoms that usually precede the diagnosis of cancer.[5] MRI and CSF examination help to exclude other diagnoses. Detection of paraneoplastic antibodies will direct a tumor search that should include the lung, breasts, and testicles in the absence of paraneoplastic antibodies.

Antineuronal Antibodies

In 60% of patients with PLE, antineuronal antibodies are found.[5,27] These include anti-Hu (36%), anti-Ta (20%), and anti-Ma (4%) antibodies.[5] Patients with anti-Ta antibodies show reactivity to Ma2 only, whereas patients with anti-Ma antibodies harbor additional reactivity with Ma1 and Ma3. Patients with anti-Ma antibodies develop additional cerebellar and more intense brainstem symptoms and have tumors other than testicular cancer.[29] Approximately 10% of patients with anti-Hu–associated PNS present with limbic encephalitis.[30–32] More recently, voltage-gated potassium channel (VGKC) antibodies were demonstrated in 4 of 15 patients with both idiopathic and paraneoplastic limbic encephalitis.[33]

Differential Diagnosis

In patients without known cancer, the differential diagnosis includes primary degenerative dementia, viral encephalitis, Creutzfeldt-Jakob disease, primary tumors (including CNS lymphoma), and psychiatric disorders. In patients with known cancer, cerebral and leptomeningeal metastases, metabolic or toxic encephalopathies, and disseminated intravascular coagulation (DIC) are the main considerations.[5]

Treatment and Prognosis

Spontaneous complete recovery has been described, albeit very rarely.[25,34] Immunotherapy is largely ineffective,[5] but multiple cases benefiting from antitumor treatment have been reported.[5,25,29] Therefore, all efforts should be directed at identifying and treating the underlying tumor. If no tumor is found, the search should be repeated every 3 months for 2 to 3 years. Irrespective of treatment, partial neurologic recovery was seen in 38% of anti-Hu, 30% of anti-Ta (Ma2), and 64% of patients without antibodies.[5]

Encephalomyelitis

Henson and colleagues introduced the term *encephalomyelitis with cancer* to define patients with clinical or pathologic evidence of severe and multiple involvement of two or more areas of the nervous system, excluding the neuromuscular junction or muscle.[35] In PEM, at least one area of the central nervous system must be involved, including

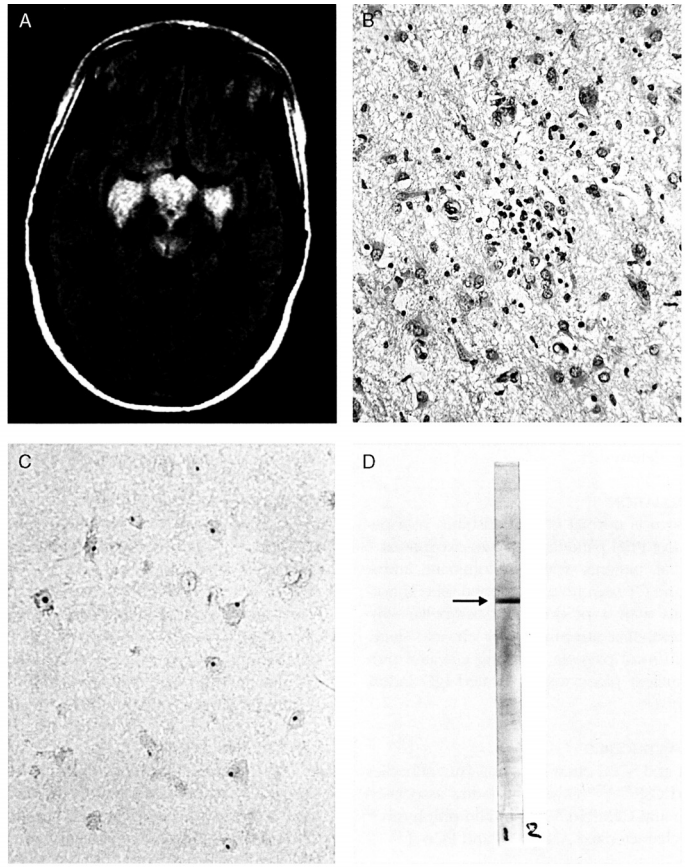


Figure 30-2 (PLATE 61). Paraneoplastic limbic encephalitis and anti-Ma2 antibodies. (A) MRI shows increased signal intensities in the medial temporal lobes, diencephalon and brainstem (FLAIR). (B) At autopsy, the medial temporal lobe showed parenchymal inflammatory infiltrates, consisting mostly of mononuclear cells. (C) The patient's serum, when reacted with human cortex, showed strong immunoreactivity against neuronal nucleoli. (D) The patient's serum (lane 1) contains immunoreactivity against purified Ma2 fusion protein (arrow). Control serum (lane 2) is negative.

the temporal lobes and cortex (limbic encephalitis), brainstem (brainstem encephalitis), cerebellum (subacute cerebellar ataxia), or spinal cord (motor neuronopathy).[10,36] Peripheral involvement affects the dorsal root ganglia (subacute sensory neuronopathy), peripheral nerves (sensorimotor polyneuropathy), or myenteric plexus (autonomic neuropathy). Patients with predominant involvement of one area but clinical evidence of only mild involvement of other areas are usually classified according to the predominant clinical syndrome. Symptoms of paraneoplastic *limbic encephalomyelitis* and *subacute cerebellar ataxia* are described above. Symptoms of *brainstem encephalitis* can include diplopia, dysarthria, dysphagia, gaze abnormalities (nuclear, internuclear, or supranuclear), facial numbness,

and subacute hearing loss. The symptoms of *myelitis* usually result from inflammatory degeneration of anterior horn lower motor neurons. Symptoms of *subacute sensory neuronopathy* and *autonomic neuropathy* are described below.

Underlying Tumor

A lung tumor is identified in 85% of PEM patients, which is SCLC in 60%.[10,30] However, a wide range of other tumors is associated less frequently with PEM.[10,30] When anti-Hu antibodies are detected, or when the patient is at risk for lung cancer (smoking, age older than 50 years), a careful and repeated search for an underlying SCLC is warranted. When CT scan and bronchoscopy are negative, a total body FGD-PET scan might detect the neoplasm.[37,38] Tumors other than SCLC detected in a patient with anti-Hu antibodies may unexpectedly express the Hu-antigen,[30] or may be unrelated secondary neoplasms.[31] When the tumor tissue is available for analysis and expresses the Hu antigen, a further workup for a second tumor (SCLC) can probably be safely deferred.[30]

Diagnostic Evaluation

MR or CT brain scan is normal or demonstrates nonspecific changes in most PEM patients with two exceptions.[36] In 65% to 80% of patients with predominant limbic encephalitis, MR and CT scan reveal temporal lobe abnormalities.[5,27] Patients with a predominant cerebellar syndrome develop cerebellar atrophy in the chronic stage. CSF is abnormal in most patients, showing elevated protein, mild mononuclear pleocytosis, elevated IgG index, or oligoclonal bands.[36]

Antineuronal Antibodies

Patients with PEM and SCLC often have anti-Hu antibodies in their serum and CSF.[30–32,36] Other antibodies associated with PEM include anti-CRMP5/CV2,[21] anti-amphiphysin,[39] and the less-well-characterized ANNA-3[40] and PCA-2.[23]

Differential Diagnosis

The differential diagnosis of PEM will vary with the most involved areas of the nervous system and is discussed more extensively with the individual syndromes. In cancer patients, leptomeningeal and parenchymal metastases can, like PEM, result in widespread and patchy involvement of the nervous system. CSF cytology and craniospinal MRI will generally rule out leptomeningeal metastases and structural lesions of the central nervous system. Vasculitis (systemic or confined to the nervous system), sarcoid and other granulomatous disorders, and infections and inflammatory disorders, including multiple sclerosis and related conditions, can all mimic PEM.

Treatment and Prognosis

Tumor treatment offers the best chance of stabilizing the patient's neurologic status, whereas immunotherapy does not appear to modify the outcome of PEM.[30,32,41] Because of incidental reports of spectacular neurologic improvement following various forms of immunosuppressive treatment, a trial of one or two immunosuppressive modalities may be warranted in a single patient. The overall functional outcome is bad, and more than 50% of patients are confined to bed or chair in the chronic phase of the disease.[30,32,41] In one study, the only independent predictor of improvement or stabilization of PEM was antineoplastic treatment.[30] Another study also identified younger age and better functional status at diagnosis as predictors of improved functional outcome.[32] The median survival of patients is approximately 1 year from diagnosis.[30,32] Mortality is predicted by worse functional status at diagnosis, age greater than 60 years, involvement of more areas of the nervous system, and absence of treatment.[30] The median time from onset of symptoms to diagnosis is approximately 4 months, and is shorter in patients with a poor functional status. Early diagnosis is facilitated by demonstration of paraneoplastic antibodies and is important to arrest the disease process before more damage is done.

Opsoclonus–Myoclonus

Opsoclonus consists of involuntary, arrhythmic, multidirectional, high-amplitude conjugate saccades and is often associated with diffuse or focal myoclonus and truncal titubation with or without cerebellar signs.[2,42] An excessive startle response reminiscent of hyperekplexia may also occur in opsoclonus–myoclonus (OM).[43] OM occurs primarily in children as a self-limited illness and is probably the result of a viral infection of the brainstem. OM may be paraneoplastic (POM) in children with neuroblastoma.

Underlying Tumor

In adult cancer patient, POM is a rare symptom. Conversely, approximately 20% of patients with OM may have a previously undiscovered malignancy.[42] The most commonly associated neoplasms are SCLC and breast and gynecologic cancers.[4,43] Many other tumors, including thyroid cancer and bladder cancer, have been reported in association with POM.[44] Approximately 2% to 3% of children with neuroblastoma have POM.[45,46]

Diagnostic Evaluation

CT and MRI are normal, while examination of the CSF may show mild pleocytosis and protein elevation. In some patients, POM resembles PCD. The prominent opsoclonus and truncal, rather than appendicular, ataxia distinguish this syndrome from anti-Yo– and anti-Hu–associated PCD.[2] Patients with POM are older (median age: 66 years) than patients with idiopathic OM (median age: 40 years).

Antineuronal Antibodies

Specific antibodies are found in only a minority of patients with POM.[4] In adults, anti-Ri antibodies are mostly associated with breast cancer and gynecologic tumors. Anti-Ri

occasionally has been found in bladder cancer and SCLC, and may occur in male patients.[2,44] POM can also be associated with anti-Hu antibodies, usually as part of a more widespread PEM. Bataller and associates[47] screened a brainstem complementary DNA (cDNA) library with sera from 21 patients with OM or POM. Twenty-five proteins were identified, recognized by one or two sera each, demonstrating that immunity to neuronal autoantigens in OM is both frequent and heterogenous.

In children presenting with OM, the detection of anti-Hu antibodies is diagnostic of an underlying neuroblastoma.[48] Combining several studies, the frequency of anti-Hu antibodies in neuroblastoma with POM is approximately 10%.[48–50] This finding differs little from the 4% to 15% of anti-Hu positive sera in children with neuroblastoma who do not have POM.[48,49]

Differential Diagnosis

The differential diagnosis of OM includes structural lesions in the posterior fossa, infections and other inflammatory conditions, including multiple sclerosis, and toxic and metabolic disorders. POM is more likely than idiopathic OM in patients older than 50 years of age who have additional encephalopathy.[4]

Treatment and Prognosis

In contrast to most of the other paraneoplastic syndromes, POM may remit either spontaneously, following treatment of the tumor, or in association with clonazepam or thiamine treatment. Most patients with idiopathic OM make a good recovery that seems to be accelerated by steroids or IVIg. Usually POM has a more severe clinical course, and treatment with steroids or IVIg appears ineffective. In a series of 14 patients with POM, 8 patients whose tumors were treated showed complete or partial neurologic recovery. In contrast, 5 of 6 patients whose tumors were not treated died of POM despite steroids, IVIg, or plasma exchange.[4]

In children, immunotherapy of POM includes corticotropin (ACTH), prednisone, azathioprine, and IVIg.[51] Treatment of the tumor with chemotherapy is the most important predictor of good neurologic recovery.[51]

Children with neuroblastoma and POM have a better survival rate than do patients without POM. Despite the excellent survival, more than two-thirds of POM patients display some sort of developmental or neurologic abnormality.[46,51]

Stiff-Person Syndrome

Stiff-person syndrome (SPS) is a clinically, electrophysiologically, and serologically heterogeneous disorder that is paraneoplastic in a subset of patients. Typically, SPS begins insidiously with gradual onset of painful rigidity and spasms in the axial muscles, spreading slowly to proximal extremity muscles.[52] Persistent contraction of the axial muscles leads to truncal rigidity, characteristic lumbar hyperlordosis, and restriction of movement of the hips and spine. Superimposed on this background rigidity are extremely painful muscle spasms that are often precipitated by psychological factors or by auditory or tactile stimuli. The spasms disappear during sleep and with localized, spinal, or general anesthesia. Electromyogram (EMG) studies show continuous motor unit activity in agonist and antagonist muscles that disappears following diazepam injection. Clinically, three categories of patients with a less typical course have been identified. One is progressive encephalomyelitis with rigidity that is often paraneoplastic.[53] It shares features with SPS but the course is relentlessly progressive, resulting in death within a few months. Additional signs of brainstem and spinal cord dysfunction are often present. The second variant is "jerking stiff-man syndrome" with prominent additional myoclonic jerking.[54] The third clinical group is the stiff-limb syndrome.[55] Clinically, these patients have painful spasms, rigidity, and posturing of the distal limb, usually the leg. The response to treatment is less favorable, and half go on to develop brainstem or sphincter involvement.

Underlying Tumor

The most common tumors associated with SPS are lung and breast cancer, but a variety of other tumors have been reported, including thymoma, Hodgkin disease, and colon cancer.[56–58] The neurologic symptoms generally precede the diagnosis of the tumor.[57]

Diagnostic Evaluation

MRI studies are normal in the majority of patients with only some reports of spinal MRI abnormalities in patients with a stiff-limb syndrome. Half of the patients with the classic form have CSF oligoclonal bands. Progressive lymphocytosis may be seen in patients with encephalomyelitis. The diagnosis is confirmed by the characteristic EMG findings and requires a high level of clinical suspicion.

Antineuronal Antibodies

Approximately 60% of patients with classic, nonparaneoplastic, SPS have antibodies against glutamic acid decarboxylase (GAD65).[59] The majority of these have coexisting diabetes mellitus or another autoimmune disorder (hyperthyroidism, vitiligo, myasthenia gravis). Anti-amphiphysin antibodies are present in the serum and CSF from female patients with paraneoplastic SPS and breast cancer.[57] Anti-amphiphysin antibodies are not specific for SPS because they are also associated with PEM and paraneoplastic sensory neuronopathy (PSN) in breast cancer and SCLC. Anti-amphiphysin antibodies have also been reported in SCLC without an associated paraneoplastic neurologic syndrome.[39,60,61] Recently anti-gephyrin antibodies were detected in a patient with SPS and an associated mediastinal tumor.[62]

Differential Diagnosis

The differential of SPS includes tetanus, neuromyotonia (see "Neuromyotonia"), strychnine intoxication, rigid spine syndrome, dystonia musculorum deformans, generalized fibrosing myositis, and psychiatric disorders.

Treatment and Prognosis

Rigidity and spasms often respond to treatment with baclofen, diazepam, valproate, vigabatrin, and carbamazepine.[63] Responses to treatment with steroids, plasma exchange, and IVIg have been reported.[64] When all these fail, methylprednisolone and cyclophosphamide pulse therapy can be tried.[65] Patients with atypical presentations, including encephalomyelitis and stiff-limb syndrome, respond less favorably to treatment.

In patients with paraneoplastic SPS, treatment of the tumor generally improves the neurologic symptoms.[66,67]

Chorea

Vernino and associates[68] describe 16 patients with paraneoplastic chorea associated with anti-CRMP5 (anti-CV2) antibodies. In 11 patients, the chorea was the initial or most prominent symptom. The chorea may be asymmetric and affects the face in most patients. Dystonic posturing is noted in a minority. Fourteen patients (88%) had other neurologic manifestations, including loss of vision as a consequence of optic neuritis (n = 5), progressive peripheral neuropathy (n = 5), limbic encephalitis (n = 5), cerebellar ataxia (n = 3), LEMS (n = 1), or myelitis (n = 1). The widespread involvement of multiple areas of the central nervous system suggests that anti-CRMP5-associated chorea is part of PEM.

Underlying Tumor

Paraneoplastic chorea is closely associated with SCLC but has also been reported with lymphoma and renal cell carcinoma.[68–70]

Diagnostic Evaluation

Most patients (83%) have mild CSF abnormalities, including lymphocytosis and elevated protein levels. Cranial MRI shows increased T_2 and FLAIR signals in the caudate and putamen which may resolve. MRI is essential to rule out other causes of chorea.

Antineuronal Antibodies

In 12% of patients with anti-CRMP5/CV2 antibodies the basal ganglia are involved, manifesting as chorea (10%), athetosis, or parkinsonism (1%).[21,68] Chorea has also been described in pathologically proven anti-Hu–associated PEM in a patient with SCLC.[71]

Differential Diagnosis

Autoimmune forms of chorea occur following streptococcal infections (Sydenham chorea) and as rare manifestations of systemic lupus erythematosus and antiphospholipid syndrome.[72] Hereditary neurologic disorders (Huntington disease, benign familial chorea, familial paroxysmal choreoathetosis, dentatorubral-pallidoluysian atrophy [DRPLA], Wilson disease, neuroacanthosis), vascular causes (basal ganglia infarction or hemorrhage, polycythemia vera, essential thrombocythemia, vasculitis), metabolic and hormonal causes (e.g., oral contraceptives, pregnancy, thyrotoxicosis, hypocalcemia, kernicterus), toxic causes (many drugs and carbon monoxide can cause chorea), and, rarely, tumors of the basal ganglia must be excluded.

Treatment and Prognosis

Chorea improves in half of patients with symptomatic treatment and in two-thirds of the patients following either chemotherapy for SCLC or intravenous methylprednisolone.[68]

Cancer- and Melanoma-Associated Retinopathy

Autoantibodies have defined two paraneoplastic visual disorders related to small cell lung cancer: retinopathy (antirecoverin antibodies) and optic neuritis (anti-CRMP5/CV2 antibodies).[73] The disorders are discussed separately in the following sections.

Cancer-associated retinopathy (CAR) results from dysfunction of the retinal photoreceptors. The incidence is equal among women and men. CAR is a subacute, progressive, autoimmune retinopathy in which the patient develops bilateral, but often asymmetric, loss of visual acuity and impaired color vision. Many patients develop night blindness and may have positive symptoms such as glaring, distortions, sparkles, and shimmering or bizarre images.

Melanoma-associated retinopathy (MAR) is a related entity in which bipolar cell function is affected.[74] Patients with MAR typically present with sudden onset of night blindness and flickering or pulsating photopsias followed by progressive loss of vision.[75] Unlike CAR, MAR is more common in men than in women.

Underlying Tumor

SCLC is the tumor most frequently associated with CAR, followed at a distance by breast cancer, non-SCLC, a variety of gynecologic tumors, and colon cancer.[76] CAR usually heralds the onset of a malignancy with an interval varying from months to 2 years or more. Conversely, MAR commonly presents after the melanoma is diagnosed with a mean latency of 3.6 years.[75]

Diagnostic Evaluation

The responses in the electroretinogram (ERG) are typically reduced as a consequence of loss of the rod and cone cells. Perimetry may show paracentral and midperipheral scotomas. Cerebrospinal fluid reveals a nonspecific mild

lymphocytic pleocytosis and an increased concentration of protein.

Antineuronal Antibodies

Many CAR patients have antibodies against the 23-kDa calcium-binding protein recoverin.[77] Approximately 90% of patients with antirecoverin antibodies harbor a SCLC. Many other autoantibodies have been described in patients with CAR, including antibodies against the 70 kDa heat-shock cognate protein, enolase-α, polypyrimidine tract-binding (PTB)-like protein, and photoreceptor cell-specific nuclear receptor. Sera from patients with MAR contain antibodies reactive with bipolar cells and other retinal elements.[74,75]

Differential Diagnosis

The differential diagnosis includes metastatic tumor and side effects of antitumor treatment. Breast, lung, and gastrointestinal carcinomas metastasize most commonly to the eye, usually to the choroid. In patients suspected of MAR, melanoma metastasis should be excluded. Approximately one-third of patients have no history of a primary tumor at the time of ocular diagnosis.

Treatment and Prognosis

Spontaneous recovery of CAR has not been reported and the symptoms generally deteriorate over weeks to months, leaving the patient with severely impaired vision. Stabilization and minimal visual improvement has been reported following immunotherapy with steroids, plasma exchange or IVIg.[78] However, once cell death has occurred, therapy is no longer of any benefit. Treatment of the tumor does not alter the course of the neurologic dysfunction in most cases. In a Lewis rat model of CAR, the retinopathy could be improved by administration of a calcium antagonist.[79] In MAR, treatment with IVIg or cytoreductive surgery (metastasectomy), improves vision in some cases.[75]

Optic Neuritis

All patients present with painless subacute loss of vision that becomes bilateral in weeks to months.[73] Bilateral optic disc swelling and nerve fiber layer hemorrhages are common.[73,80] Some patients develop concomitant symptoms of myelopathy, superficially resembling Devic disease. In the majority of patients, abundant cells are present in the posterior vitreous. Fluorescein angiography shows vascular leakage at and remote from the disc. In addition, most tested patients have abnormal retinograms. Vitrectomy shows reactive lymphocytosis, predominantly CD4+. Most patients presenting with optic neuritis develop a variety of other neurologic symptoms suggestive of PEM.

Underlying Tumor

In a series of 16 anti-CRMP5–positive patients with paraneoplastic optic neuritis, 69% had SCLC whereas in 2 patients no active cancer was detected.[73] Renal cell and thyroid carcinoma appear associated with the Devic-like disease.[73]

Antineuronal Antibodies

Anti-CRMP5/CV2 antibodies are associated with paraneoplastic optic neuritis and SCLC.[21,22,73] Demonstration of anti-CRMP5/CV2 antibodies obviates the need of vitrectomy to exclude ocular lymphoma and directs the search of an underlying malignancy to the lung.

Treatment and Prognosis

Treatment of the tumor may result in stabilization or even improvement of the optic neuritis.[81,82] Steroids alone may also improve vision in paraneoplastic optic neuritis.[83]

Subacute Motor Neuronopathies/Motor Neuron Disease

Weakness without sensory symptoms can be caused by paraneoplastic disorders that involve the lower motor neuron anywhere along its course from the anterior horn cells to the neuromuscular junction. Although some axonal and demyelinating paraneoplastic neuropathies are clinically predominantly motor, they are considered below under "Sensorimotor Neuropathy." Although most associations between motor neuron disease (MND) and cancer are based on chance, some syndromes may be paraneoplastic. A paraneoplastic etiology is suggested (but not proven) by common association of a characteristic motor neuron syndrome with a typical tumor, by improvement of MND following treatment of the tumor, and by the association with an onconeural antibody.[84]

Characteristic Associations of Motor Neuron Syndromes with a Typical Tumor

A disorder called subacute motor neuronopathy is associated with Hodgkin and non-Hodgkin lymphoma and may be a variant of MND.[85,86] Characteristically, a patient already known to harbor a lymphoma develops subacute painless and often patchy lower motor neuron weakness. The proximal legs are more affected than the arms, while the bulbar muscles usually remain spared. Atrophy is present, but fasciculations are not prominent. Upper motor neuron and sensory signs are usually absent. Although the disease can result in death, the course is usually benign and independent of the activity of the underlying neoplasm. Unlike MND, the neurologic defect often stabilizes or improves spontaneously after months or years without incapacitating the patient. Electroneurophysiology demonstrates signs of denervation with intact conduction velocities. Pathology shows loss of anterior horn motor neurons while the lateral columns of the spinal cord, unlike in MND, are typically spared. Indirect evidence suggests a viral etiology but a specific virus has never been isolated.

A different lower motor neuron syndrome can occur as a complication of radiotherapy to the spinal cord. In this disorder, the legs are affected with more distal than proximal weakness. Spontaneous improvement does not occur.[87,88] A slowly evolving lower motor neuron syndrome may also complicate mantle irradiation for Hodgkin lymphoma. These patients develop weakness and atrophy of predominantly proximal muscles including the neck, paraspinal, and shoulder musculature.

One prospective series of 37 MND patients shows a higher-than-expected incidence of lymphoma.[89] The main clinical implication is that patients with MND should probably be screened by immunofixation for the presence of a paraprotein. Bone marrow biopsy should be considered in patients with paraproteinemia, CSF oligoclonal bands, or an increased CSF protein (>0.75 g/L).

The third association is an upper motor neuron syndrome resembling primary lateral sclerosis in patients with breast cancer.[90] The symptoms usually develop in close temporal relationship (months) with the tumor diagnosis. The syndrome is slowly progressive and some patients remain free from lower motor neuron involvement.

Improvement of MND Following Treatment of the Tumor

MND, including amyotrophic lateral sclerosis (ALS), has been reported in association with cancer.[91,92] Systematic reviews suggest that the association is coincidental in most patients.[91] However, reports of stabilization, or even remission, of motor neuron symptoms in some patients,[1,93] and unusual pathologic findings, including inflammatory changes in others,[94] suggest that some cases of MND may have a paraneoplastic origin.

MND Associated with Paraneoplastic Antibodies

Predominant motor neuron dysfunction can occur as part of PEM.[35] When the underlying tumor is a SCLC, the anti-Hu antibody is often present.[30–32,36,84] In these patients, the symptoms have an acute or subacute onset with rapid progression and eventual involvement of other areas of the nervous system, differentiating this syndrome from ALS. Incidentally, other antibodies have been described in combination with pure motor syndromes. One patient developed ophthalmoplegia and subacute motor axonal neuropathy in association with anti-GQ1b antibodies and recurrent malignant melanoma. Response to immunotherapy (steroids) and expression of the GQ1b antigen in her tumor strongly suggested a paraneoplastic etiology.[95] Berghs and associates[96] reported the case of a woman with breast cancer and a paraneoplastic lower motor neuron syndrome whose serum contained autoantibodies directed against axon initial segments and nodes of Ranvier of myelinated axons, including the axons of motor neurons. The major targets of these autoantibodies were beta-IV spectrin isoforms.

Peripheral Nervous System

Subacute Sensory Neuronopathy

The clinicopathologic description of PSN was established by Denny-Brown in 1948.[97] He first described two patients with "spreading numbness and sensory loss of the extremities (in one the face was also involved) and severe and progressive ataxia associated with carcinoma." The pathologic hallmark is reflected in the name and consists of inflammatory infiltrates with neuronal cell loss in the dorsal root and corresponding cranial nerve (Gasserian) ganglia.[97,98] Although all ganglia are affected, the distribution may be patchy. Subacute sensory neuronopathy is an uncommon disorder of middle age, equally affecting males and females. Subacute sensory neuronopathy is probably paraneoplastic in approximately 20% of patients.[3,99] The symptoms begin with painful paresthesias and dysesthesias. Clumsiness and unsteady gait then develop and usually become predominant. Initially symptoms are usually distal and may be very asymmetric, although sometimes the distribution is more proximal in the limbs, trunk, and, occasionally, the face. In most patients, the disease progresses rapidly over weeks to months, leaving the patient severely disabled. In a few patients, the neuronopathy remains stable for months, with mild neurologic deficits.[100] On examination, all sensory modalities are affected, but the most striking abnormality is sensory ataxia with pseudoathetosis of the hands. Tendon reflexes are depressed or absent.

Underlying Tumor

Two-thirds of patients with PSN have lung cancer, in particular SCLC.[3,31,36] Other associated tumors include breast cancer and Hodgkin disease.[3,99] Usually the neuronopathy precedes the tumor diagnosis by an average of about 1 year.

Diagnostic Evaluation

Electrophysiologic studies demonstrate small or absent sensory action potentials in the presence of normal motor nerve conduction velocity and F waves. Early in the course of the disease there is a mild pleocytosis, with an elevated IgG and oligoclonal bands.[3,32,99] Assessment of paraneoplastic antineuronal antibodies in the serum of patients with subacute sensory neuronopathy is a useful test.

Antineuronal Antibodies

Associated paraneoplastic antibodies include anti-Hu, anti-amphiphysin, and anti-CRMP5/CV2.[6,21,30–32,36,39] The most frequent antineuronal antibody in PSN is anti-Hu.[30–32,36] In a series of 126 patients presenting with

subacute sensory neuronopathy, anti-Hu antibodies were detected in 40 patients (32%) with PSN. One patient with anti-Hu antibodies had idiopathic sensory neuronopathy. The specificity of anti-Hu detection was 99% and the sensitivity was 82%.[101]

Differential Diagnosis

The pain and marked asymmetry in the beginning may lead to a diagnosis of (poly)radiculopathy or mononeuritis multiplex.[3] PSN is not always a purely sensory syndrome, and patients may demonstrate various involvement of motor nerves, autonomic nervous system, spinal cord, or brain (paraneoplastic encephalomyelitis). In a patient without cancer, the differential diagnosis of subacute sensory neuropathy includes collagen-vascular disease (e.g., Sjögren syndrome), idiopathic sensory neuronopathy, toxins, and primary biliary cirrhosis.[102] For patients undergoing active cancer treatment, the main differential diagnosis is chemotherapy-induced neuropathy, particularly by cisplatin and taxanes.[103]

Treatment and Prognosis

The course of the disorder is mostly independent of the tumor; neurologic symptoms usually progress rapidly and then stabilize. Immunotherapy consisting of plasma exchange, steroids, IVIg, and cyclophosphamide is ineffective in most cases.[41] Nevertheless, individual case reports suggest that some patients may benefit from immunotherapy. Treatment of the underlying neoplasm, usually SCLC, often results in complete remission of the tumor. On average, patients receiving antitumor treatment have a better neurologic outcome.[104] Interestingly, patients with SCLC and anti-Hu antibodies respond better to antitumor treatment than do SCLC patients without a paraneoplastic syndrome.[105] In patients with an identifiable tumor, antitumor treatment is recommended. In the absence of a tumor, antitumor treatment may be considered in patients with anti-Hu antibodies, age older than 50 years, and a history of smoking. In patients not receiving antitumor therapy, a short course of immunotherapy can be considered. Based on the literature, it is impossible to recommend one form of immunotherapy over another.

Sensorimotor Neuropathy

Paraneoplastic sensorimotor neuropathies are clinically and electrophysiologically heterogenous and are associated with many different tumor types.[106]

Because of this heterogeneity the association of the tumor and the neuropathy will be coincidental in many cases. A relatively mild sensorimotor neuropathy develops in approximately 15% of cancer patients in the terminal stage of the disease and may be related to cachexia.[106] Another group of paraneoplastic neuropathies is much more severe and develops rapidly, often before the cancer diagnosis is made. Antoine and associates[6] studied 26 patients with a severe neuropathy preceding the tumor diagnosis in most patients. Seven patients had paraneoplastic antibodies (6 anti-Hu; 1 anti-CRMP5/CV2), whereas 19 did not. All six patients with anti-Hu antibodies had signs of sensory neuronopathy, which was combined with motor neuropathy in two. Electrophysiologic studies were consistent with a mild axonal motor neuropathy in three of the six patients. In fact, these patients can better be classified as sensory neuronopathy with motor involvement. The patient with anti-CRMP5/CV2 antibodies had a mild axonal motor neuropathy. The 19 antibody-negative patients could be divided into four groups: (a) four patients with sensory neuronopathy or sensory neuronopathy with motor involvement as part of rapidly progressive PEM; (b) two patients with mononeuritis multiplex who had systemic or nerve-restricted nonnecrotizing vasculitis; (c) seven patients with an axonal sensorimotor polyneuropathy without inflammatory changes in their nerve biopsies and who showed stabilization after several months or years of followup; and (d) six patients with a demyelinating neuropathy, which was acute in one patient, who presented as a typical Guillain-Barré syndrome. The other patients had a CIDP-like neuropathy.[7]

Associated Tumors

The associated tumors are very heterogenous.

Paraneoplastic Antibodies

Anti-Hu antibodies and anti-CRMP5/CV2 are most commonly associated with sensorimotor neuropathies.[6,30–32,36,107] Of 20 patients with anti-Hu antibodies and predominant peripheral neuropathy, 14 (70%) had sensory neuronopathy, 5 (25%) had sensorimotor neuropathy, and 1 (5%) had motor neuronopathy.[108] The electrophysiologic patterns in the five patients with sensorimotor neuropathy were sensory axonal/neuronal and motor mixed axonal/demyelinating.

Differential Diagnosis

In patients with cancer, the development of a peripheral neuropathy usually represents a side effect of therapy (taxanes, vinca alkaloids, cisplatin), infiltration of nerves or spinal roots by the tumor, or metabolic and nutritional deficits. The diagnosis of a rapidly progressive debilitating neuropathy is a challenge for the neurologist and requires extensive examinations. Demonstration of paraneoplastic antibodies defines the neuropathy as paraneoplastic and directs the search to an underlying neoplasm.

Sensorimotor Neuropathy Associated with M Proteins

The malignancies that are associated with malignant monoclonal (or M) proteins include disorders that produce

IgG or IgA proteins (osteosclerotic myeloma and multiple myeloma) and disorders that produce IgM proteins (Waldenström macroglobulinemia, B-cell lymphoma, and chronic B-cell lymphocytic leukemia).

Osteosclerotic Myeloma

Osteosclerotic myeloma is a rare (1%–2%) and relatively benign variant of multiple myeloma. The disorder is characterized by single or multiple sclerotic bone lesions that are commonly located in the long bones of the arms and legs, the spine, or the pelvis. The lesions are not found in the skull where osteolytic lesions commonly occur in typical myeloma. More than 50% of patients with osteosclerotic myeloma develop a characteristic polyneuropathy.[109,110] Deficits are predominantly motor and often slowly progressive over months to years. The neuropathy is sometimes mistaken for a slowly progressive form of CIDP. All or some features of the POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, and skin changes) syndrome can be present. Correct diagnosis is important because these patients may improve with treatment of the myeloma. Patients with single lesions do best. Radiation or excision of the osteosclerotic lesion results in the elimination of the M protein and in gradual recovery in the ensuing months. Patients with multiple lesions are more difficult to treat and generally require chemotherapy (usually consisting of alkylating agents combined with high doses of steroids).[111] Immunotherapy is generally ineffective in these patients.

Multiple Myeloma

Although approximately 30% of patients with multiple myeloma have electrophysiologic signs of peripheral neuropathy, only a few percent develop clinical symptoms.[112–114] The neuropathy usually precedes the diagnosis of the myeloma. Weakness and sensory symptoms in the distal limbs appear over a period of weeks. In typical cases, neurophysiologic studies demonstrate axonal damage whereas biopsies often show destruction of both axons and myelin. Approximately 30% to 40% of patients with multiple myeloma and neuropathy develop amyloid deposits. In these patients, atypical features including carpal tunnel syndrome, mononeuritis multiplex, and autonomic features occur, superimposed on the symptoms of mild axonal sensorimotor neuropathy. Sensory neuropathy and a CIDP-like neuropathy are also associated with multiple myeloma. No specific treatment exists for these neuropathies, and treatment of the myeloma does not influence the neurologic symptoms.

Waldenström Macroglobulinemia

Waldenström macroglobulinemia may arise from IgM–MGUS (monoclonal gammopathy of unknown significance), and similar polyneuropathy syndromes affect these two groups of patients. Approximately 10% of patients with Waldenström macroglobulinemia develop a symptomatic polyneuropathy.[115] The neuropathies can be separated into demyelinating polyneuropathy associated with IgM anti-MAG (myelin-associated glycoprotein) antibodies, demyelinating polyneuropathies with IgM antibodies reactive to gangliosides (but not with MAG), polyneuropathies with monoclonal IgM proteins nonreactive with a known antigen, cryoglobulinemic neuropathy, and amyloid polyneuropathy. Approximately 50% of patients with Waldenström macroglobulinemia and a demyelinating polyneuropathy have IgM anti-MAG antibodies and present with a progressive distal symmetric sensorimotor polyneuropathy. Predominant involvement of large sensory fibers results in postural tremor and pseudoathetosis. The CSF often shows increased protein concentration, and electrophysiologic studies demonstrate slow conduction velocities and prolonged distal motor and sensory latencies. Biopsies show widening between lamellae of myelin sheaths caused by intercalation of anti-MAG antibodies. The treatment should be directed at the tumor and symptomatic sensorimotor neuropathy may be reason to start treatment of Waldenström macroglobulinemia.[116,117] Occasionally, patients with IgM anti-MAG respond to treatment with plasma exchange or IVIg. A promising new agent for polyneuropathies associated with IgM M proteins is the anti-CD20 monoclonal antibody rituximab.[118,119]

Subacute Autonomic Neuropathy

In a series of 157 patients presenting with symptoms of autonomic failure, 18 (12%) had paraneoplastic subacute autonomic neuropathy.[120] In subacute autonomic neuropathy, sympathetic failure is manifested as severe orthostatic hypotension and anhydrosis, and parasympathetic failure as dry mouth, sexual dysfunction, impaired pupillary response to light and accommodation, and a fixed heart rate. Many patients have gastrointestinal dysmotility varying from anorexia and early satiety to diarrhea or chronic pseudo-obstruction. In LEMS, autonomic signs or symptoms are ultimately present in 80%.[121]

Underlying Tumor

In 12 of 18 patients with paraneoplastic subacute autonomic neuropathy, the underlying tumor was SCLC. Ten of these patients had anti-Hu antibodies.[120] Other tumors included thymoma in four and rectal and bladder cancer in one. In a series of 12 patients with paraneoplastic gastric motility disorders, 9 had an underlying SCLC, and 8 of these harbored anti-Hu antibodies.[122]

Antineuronal Antibodies

Vernino and associates found anti-Hu antibodies in 56% of patients with paraneoplastic subacute autonomic

neuropathy. Previously, autonomic involvement has been reported in up to 25% of patients with PEM/SN and anti-Hu antibodies.[30–32,36] Autoantibodies that bind or block specifically with the nicotinic acetylcholine receptors in the autonomic ganglia are present in 40% of patients with idiopathic or paraneoplastic subacute autonomic neuropathy.[120]

Differential Diagnosis

The differential diagnosis includes idiopathic subacute autonomic neuropathy, postural tachycardia syndrome, idiopathic gastrointestinal dysmotility, diabetic and amyloid autonomic neuropathy, nondiabetic sensorimotor and autonomic neuropathy, pure autonomic failure, and multiple system atrophy.[123] In familial cases, a form of hereditary sensory and autonomic neuropathy (HSAN) I to V should be excluded.[123]

Treatment and Prognosis

The disease is usually monophasic with slow and incomplete or no recovery. Stabilization may be achieved by treatment of the tumor.[124] Palliation of the debilitating symptoms of pseudo-obstruction may be achieved by repeated administration of neostigmine.[125]

Vasculitis of Nerve and Muscle

Patients often present with a painful asymmetric or symmetric polyneuropathy or, less commonly, with a mononeuritis multiplex. The neuropathy may antedate the diagnosis of the neoplasm. The neuropathy is subacute and progressive and usually affects older men.[126] Some patients with paraneoplastic vasculitic neuropathy also suffer from LEMS or PEM.

Underlying Tumor

A localized vasculitis of the nerve and muscle is found most commonly with lung cancer and lymphomas.[126] It is also reported in association with malignancies of prostate, breast, endometrium, kidney, and bile duct. In 50% of patients, the vasculitic symptoms precede the cancer diagnosis.

Diagnostic Evaluation

Two helpful laboratory abnormalities are a high erythrocyte sedimentation rate and high CSF protein content. Electrophysiologic studies show axonal degeneration involving both sensory and motor nerves. The diagnosis is confirmed by a nerve or muscle biopsy showing intramural and perivascular inflammatory infiltrates that are mainly composed of CD8+ T-lymphocytes.[127]

Antineuronal Antibodies

When the vasculitic neuropathy is associated with SCLC, anti-Hu antibodies have been described.[36]

Treatment and Prognosis

Recognition of this syndrome is important, because the disorder may respond to both antineoplastic and immunosuppressive treatment. Combination of steroids and cyclophosphamide may offer better results than steroids alone.[126]

Neuromyotonia

Acquired neuromyotonia (Isaac syndrome, cramp-fasciculation syndrome, undulating myokymia) is characterized by spontaneous and continuous muscle fiber activity. Symptoms include muscle cramps in proximal and distal muscles, made worse by exercise and stiffness. Examination reveals myokymia, pseudomyotonia (slow relaxation) and excessive sweating. The rippling and twitching of muscles sometimes lead to sustained abnormal posture. Paraneoplastic neuromyotonia can be part of PEM. In patients with neuromyotonia, other autoimmune disorders such as myasthenia gravis occur more frequently than expected.[128]

Underlying Tumor

In a series of 60 patients with neuromyotonia, an underlying neoplasm was identified in 15 (25%).[128] Paraneoplastic neuromyotonia is most often associated with thymoma or lung cancer.[128] Other reported tumors include Hodgkin lymphoma and plasmocytoma.

Diagnostic Evaluation

EMG shows fibrillation, fasciculation, myokymic discharges, doublets, triplets, and high-frequency neuromyotonic discharges.[129] This abnormal muscle activity continues during sleep and general anesthesia, is abolished by curare, and may be unaffected or reduced by peripheral nerve block.[130]

Antineuronal Antibodies

Both paraneoplastic and idiopathic neuromyotonia are associated with autoantibodies to VGKC.[128] This antibody increases the release of quanta of acetylcholine and prolongs the action potential, which may explain the peripheral nerve hyperexcitability in the patients.[131,132]

Treatment and Prognosis

Symptoms of neuromyotonia may improve with carbamazepine, phenytoin, or other antiepileptic drugs.[129] When the disease is immune-mediated, steroids, plasma exchange, and IVIg may be effective.[129] Because of the small number of reported cases, the impact of tumor treatment on neuromyotonia is not known.

Differential Diagnosis

Muscle cramps are common complications of cancer and are most often related to electrolyte imbalances. Disorders

of the central nervous system such as stiff-person syndrome, tetanus, and neuroleptic malignant syndrome can be confused with neuromyotonia. Stiff-person syndrome is characterized by prominent rigidity and continuous contraction of paraspinal muscles with intermittent paroxysmal painful muscle spasms. The distinguishing feature is the disappearance of abnormal muscle activity during sleep or anesthesia with stiff-person syndrome (but not with neuromyotonia).

Neuromuscular Junction and Muscle

Lambert-Eaton Myasthenic Syndrome

Most patients present with proximal weakness of the lower extremities and fatigability, but unlike myasthenia gravis, the symptoms do not significantly affect the bulbar musculature. Respiratory weakness can occur. Deep tendon reflexes, especially those in the legs, are diminished or absent but may reappear after exercise. Autonomic features, particularly dry mouth, impotence, and mild/moderate ptosis, ultimately develop in 80%.[121,133] In some patients, LEMS may develop in association with other paraneoplastic syndromes, including PCD and PEM/SN.[14]

Underlying Tumor

Sixty percent of patients have a SCLC.[121] Other tumors include small cell carcinomas of the prostate and cervix, lymphomas, and adenocarcinomas. The prevalence of LEMS in SCLC is estimated to be approximately 3%.[133,134] Clinically and serologically, the 40% without identifiable tumor are indistinguishable from the paraneoplastic LEMS patients.

Diagnostic Evaluation

The typical pattern of electromyographic abnormalities is the hallmark of LEMS (Fig. 30-3). This includes a low, compound muscle action potential at rest with a decreased response at low rates of repetitive stimulation (3 Hz) and an incremental response at high rates of repetitive stimulation (50 Hz) or 15 to 30 seconds of maximal voluntary contraction.[135]

Antineuronal Antibodies

Most patients with LEMS have antibodies against VGCC that are located presynaptically in the neuromuscular junction.[135] Approximately 20% have anti-MysB antibodies reactive with the β-subunit of neuronal calcium channels.[136]

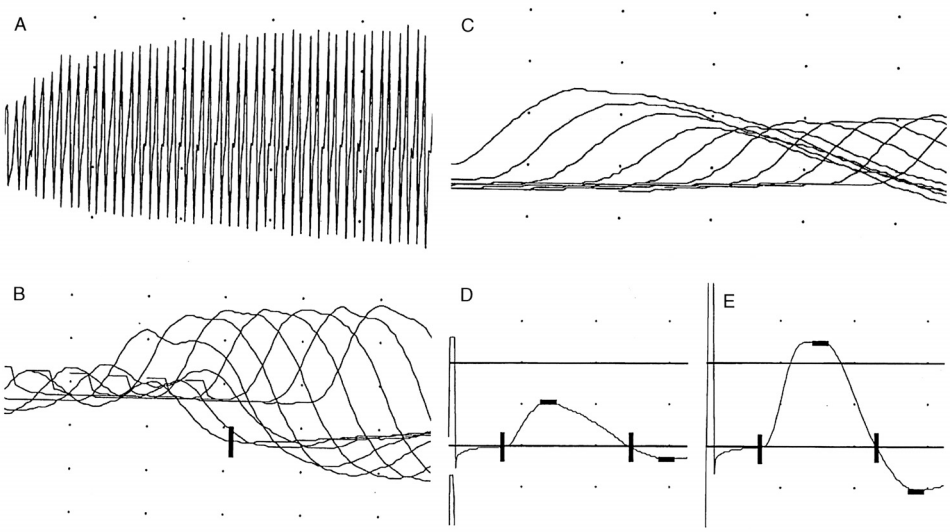


Figure 30-3. Electrophysiological testing in a patient with LEMS. (A,B) Repetitive nerve stimulation (50 Hz) of the ulnar nerve registering over the musculus abductor digiti minimi results in an increment of the amplitude of 100% between the first and the 10th stimulus. (C) Repetitive nerve stimulation at a frequency of 3 Hz results in a >50% decrement of the amplitude. (D) After a single stimulus, the CMAP of the musculus abductor digiti minimi is decreased (2.8 mV). (E) Following maximal voluntary contraction, the CMAP significantly increases (7.2 mV). (*Courtesy Marjan Scheltens, MD.*)

Differential Diagnosis

Proximal weakness in cancer patients can have many neoplastic and treatment-related causes. The characteristic clinical picture (including autonomic dysfunction) in combination with EMG findings and anti-VGCC antibodies is diagnostic of LEMS.

Treatment and Prognosis

Treatment of LEMS must be tailored to the individual based on severity of the symptoms, underlying disease, life expectancy, and previous response to treatment. In patients with paraneoplastic LEMS, treatment of the tumor frequently leads to neurologic improvement.[137] Symptomatic treatment is with drugs such as 3,4-diaminopyridine (DAP) that facilitate the release of acetylcholine from motor nerve terminals.[138] In a placebo-controlled randomized trial, DAP (5–20 mg TID-QID) was effective for long-term treatment, alone or in combination with other treatments.[139] The maximum recommended daily dose of DAP is 80 mg; at higher doses seizures occur.[139] Cholinesterase inhibitors (pyridostigmine, 30–60 mg q6h) may improve dryness of mouth but rarely relieve weakness. If these treatments are not effective enough, immunosuppressive therapy with steroids, azathioprine or cyclosporine may be considered. Removal of antibodies by plasma exchange[140] and IVIg can provide quick, but transient, relief.[121,141] LEMS responds less favorably to immunotherapy than myasthenia gravis.

Myasthenia Gravis

Myasthenia gravis (MG) is a postsynaptic disorder of neurotransmission caused by antibodies reacting with the nicotinergic acetylcholine receptors at the neuromuscular junction. In ocular MG, diplopia and ptosis as a result of weakness of extraocular and levator palpebrae muscles predominate. In generalized MG, the patient has additional facial, oropharyngeal, and limb muscle weakness. The weakness fluctuates over time and increases with repeated efforts of the affected muscles. There are no sensory symptoms or signs of denervation.

Underlying Tumor

MG occurs in approximately 30% of patients with a thymoma and, conversely, approximately 10% of patients with MG harbor a thymoma. Symptoms do not differ between patients with or without thymoma.

Diagnostic Evaluation

The diagnostic evaluation may include EMG, autoantibody assay, and pharmacologic testing. The EMG shows characteristic decrement of the compound muscle action potential (CMAP; >10%) during repetitive nerve stimulation.[142] EMG is diagnostic in 50% to 80% of patients with ocular MG and in 90% to 100% of patients with generalized MG. Autoantibodies against the nicotinic acetylcholine receptor (anti-AChR) are present in 70% to 80% of patients with ocular MG and in 90% to 100% of patients with generalized MG. Single-fiber EMG is more sensitive than repetitive nerve stimulation.[142] Temporal reversion of weakness by acetylcholinesterase inhibitors (neostigmine) is a useful diagnostic test.

Antineuronal Antibodies

Anti-AChR antibodies are present in approximately 85% of all MG patients. All MG patients with a thymoma have anti-AChR antibodies.[143] Antibodies to striated muscle proteins, including the titin protein, are present in 85% of patients with MG and thymoma, and in 30% of patients with either MG or thymoma.[144] In young patients, the anti-striated muscle antibodies help diagnose an underlying thymoma. Recently, an IgG antibody against the muscle-specific kinase (MuSK) was identified.[145] The antibody is present in approximately 40% of seronegative MG patients.[143] The presence of antibodies against MuSK defines a subgroup of patients with seronegative MG who have predominantly localized, in many cases bulbar, muscle weakness and reduced response to conventional immunosuppressive treatments. Moreover, muscle wasting may be present, which prevents complete response to these therapies.[143]

Treatment and Prognosis

Symptomatic treatment of MG relies on cholinesterase inhibitors. Immunotherapy comprises thymectomy, immunosuppressive drugs, plasma exchange, and IVIg.[143]

Differential Diagnosis

The differential diagnosis of MG includes other disorders of the neuromuscular junction (e.g., LEMS, congenital myasthenic disorders, botulism, and snakebite poisoning), MND, inflammatory neuropathies, myopathies, and oculopharyngeal muscular dystrophy.

Dermatomyositis/Polymyositis/Inclusion Body Myositis

Clinical, electromyographic, and pathologic findings of the inflammatory myopathies are similar in patients with and without cancer. The course is usually subacute and slowly progressive. In dermatomyositis, the characteristic heliotrope rash (purplish discoloration of the eyelids) often precedes the appearance of proximal muscle weakness. Other manifestations include arthralgia, myocarditis and congestive heart failure, and interstitial lung disease. Polymyositis presents with subacute or insidious weakness of neck flexors and proximal limb muscles. Involvement of distal muscles varies. Inclusion-body myositis (IBM) is characterized by a gradual but relentlessly progressing weakness of both proximal and distal muscles. A distinctive clinical feature is atrophy of the volar forearm muscles.[146]

Underlying Tumor

The most recent evidence from population-based cohort studies confirms the association between malignancy and dermatomyositis, polymyositis, and IBM.[147–149] An underlying malignancy is more common in patients older than age 50 years, but can occur in children.[150] In contrast to other more typical paraneoplastic neurologic syndromes, the underlying tumors are very heterogenous. Buchbinder and associates[147] found that the highest risk for a malignant disease is associated with dermatomyositis (standardized incidence ratio, 6.2 [95% confidence interval (CI), 3.9–10.0]). The risk is also increased in polymyositis (standardized incidence ratio, 2.0 [5% CI, 1.4–2.7]), whereas the excess risk for malignancy in IBM is 2.4 (95% CI, 2.7–7.1). The excess risk for cancer diminishes with the time from diagnosis. Dermatomyositis is associated with a higher risk for cancer of the ovary, lung, pancreas, non-Hodgkin lymphoma, stomach, colorectal, and breast.[148] In polymyositis, non-Hodgkin lymphoma and tumors of the lung and bladder are overrepresented.[148]

Diagnostic Evaluation

Most patients with dermatomyositis/polymyositis have elevated serum creatine kinase (CK) levels and electromyographic evidence of myopathy. In IBM, CK levels are lower than in polymyositis and may be normal. Usually CK levels in IBM are two- to fivefold elevated. Muscle imaging (CT or MRI) may help in confirming the diagnosis, determining the type of inflammatory myopathy, and selecting an appropriate biopsy site. Muscle biopsy is the definitive diagnostic procedure and shows inflammatory infiltrates.[150]

Associated Antibodies

Anti-Jo-1 (an antibody against histidyl-transfer RNA synthetase) is present in approximately 30% of patients with polymyositis and is associated with interstitial lung disease.[151]

Antibodies to the Mi-2 protein complex are highly specific for dermatomyositis and are present in high titers in approximately 35% of cases, including cases of juvenile dermatomyositis.[151] Antibodies to the polymyositis (PM)-Scl nucleolar antigen (anti-PM-1) are present in 50% of patients with the scleroderma-myositis overlap syndrome.[150]

Treatment and Prognosis

Treatment of paraneoplastic dermatomyositis/polymyositis is generally the same as for patients without tumor. Nearly all patients with dermatomyositis/polymyositis respond to corticosteroids.[146] Refractory patients and patients requiring steroid-sparing agents may be treated with azathioprine. Methotrexate and cyclophosphamide can also be considered.[146]

Acute Necrotizing Myopathy

Acute necrotizing myopathy manifests as muscle pain and proximal weakness. The course of the disease is rapidly progressive, involves the pharyngeal and respiratory muscles, and can lead to death within a few weeks to months.[152] Many solid tumors can accompany this syndrome, including SCLC, gastrointestinal tract cancers, and malignancies of breast, kidney, and prostate.[153] The serum creatine kinase is markedly increased, and muscle biopsy shows prominent necrosis with little or absent inflammation. No specific autoantibody is reported. Some patients improved after successful antineoplastic therapy.[153] The differential diagnosis includes polymyositis and rhabdomyolysis related to chemotherapy and combination treatment including interleukin (IL)-2 and interferon-α.[154]

Paraneoplastic Carcinoid Myopathy

Paraneoplastic carcinoid myopathy is found in only 0.5% of patients with carcinoid tumors and may present years before tumor detection. The muscle enzymes are mildly elevated, and muscle biopsy shows nonspecific changes, including type II atrophy, necrosis of fibers, central nuclei, and mitochondrial changes. Symptoms may improve with serotonin antagonists such as cyproheptadine or the somatostatin analogue octreotide.[155]

Cachectic Myopathy

Cachexia is defined as a significant involuntary weight loss that complicates debilitating diseases, including cancer. The cancer cachexia syndrome may be present in up to 80% of patients with cancer.[156] Despite diffuse muscle wasting, the strength of cancer patients with cachectic myopathy is often preserved until late in the course of the disease. Biopsy shows muscle atrophy involving both types of fibers without evidence of inflammation or nerve degeneration. Malnutrition resulting from cancer cachexia is a significant cause of morbidity and mortality.[156] Recent studies suggest that growth factors and host cytokines produced in response to the tumor play a role in the pathogenesis of paraneoplastic cachexia.[156] The host cytokines include tumor necrosis factor-α, IL-1, IL-6, interferon-γ, and D-factor.[156] The efficacy of nutritional support in cancer related cachexia has not been adequately studied.[156]

► PARANEOPLASTIC AUTOANTIBODIES

Many patients with paraneoplastic neurologic syndromes have in their serum (and CSF) antibodies that react with both the tumor and with neuronal elements in

► **TABLE 30-4.** TUMOR SPECIFICITY OF PARANEOPLASTIC ANTIBODIES

Well-Characterized (Typical) Paraneoplastic Antibodies	Partially Characterized Paraneoplastic Antibodies	Antibodies that Also Occur Without Cancer
Anti-Hu (ANNA-1)	Anti-mGluR1	Anti-Tr (PCA-Tr)
Anti-Yo (PCA-1)	ANNA-3	Anti-VGCC
Anti-Ri (ANNA-2)	PCA-2	Anti-VGKC
Antiamphiphysin	Anti-Zic4	Anti-nAChR
Anti-CRMP5 (anti-CV2)		Anti-MAG
Antirecoverin		
Anti-Ma/Ta		

ANNA = antineuronal nuclear antibody; MAG = myelin-associated glycoprotein; mGluR1 = metabotropic glutamate receptor type 1; PCA = Purkinje cytoplasmic antibody; VGCC = voltage-gated calcium channels; VGKC = voltage-gated potassium channel.

affected parts of the nervous system. These onconeural or paraneoplastic antibodies are most important diagnostically because of their high specificity and association with certain tumor types (see Table 30-3). In the past 15 years, many paraneoplastic antibodies have been described. The typical paraneoplastic antibodies have been studied in large series and are now well characterized (Table 30-4). The association of anti-Yo, anti-Hu, anti-Ri, anti-amphiphysin, anti-CRMP5/CV2, anti-recoverin, and anti-Ma/Ta antibodies with both cancer and severe neurologic syndromes is firmly established. In patients with these antibodies, the likelihood of developing a tumor is almost 100%. With other, more recently discovered antibodies, the association is less robust because the antibody specificity has not yet been confirmed in another laboratory or too few patients have been studied (Table 30-4). A third group consists of autoantibodies that are associated with both the idiopathic and paraneoplastic variant of the neurologic syndrome. Examples include LEMS with anti-VGCC that is associated with lung cancer in 60% of cases.[121] Anti-Tr antibodies that are associated with Hodgkin lymphoma or other tumors in 90% of cases provide another example. Ten percent of the cases are idiopathic and, in contrast to the typical paraneoplastic antibodies, the target antigen could not be demonstrated in most tumors.[18]

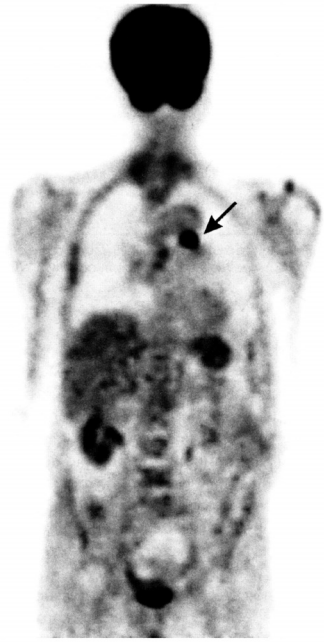
Although there is considerable overlap, each of the antibodies is associated with a restricted spectrum of clinical syndromes and tumors. In most cases, the target antigen has been cloned and identified (see Table 30-3). Expression of the onconeural antigen by both the tumor and in affected parts of the nervous system supports an autoimmune etiology. Some of the paraneoplastic antigens are expressed by all tumors of a certain histology regardless of the presence of an autoimmune response. For example, Hu antigens are expressed by most SCLC, whereas only 16% of patients will develop low-titer anti-Hu antibodies and <1% will develop high-titer antibodies and a paraneoplastic syndrome.[105,157] Other tumors rarely express onconeural antigens unless the cancer causes a paraneoplastic neurologic disorder.[158]

Unfortunately, approximately 50% to 60% of patients with a paraneoplastic syndrome have no detectable onconeural antibodies in their serum. Therefore, the absence of paraneoplastic antibodies does not exclude PNS.

The detection of paraneoplastic antibodies is carried out in specialized academic laboratories and in more limited settings using commercially available kits. The quality of commercial kits varies widely and consist of a dot-blot assay reactive with four of the most common onconeural antigens (HuD, CDR62, Nova, Amphiphysin). However, the wide variety of clinical findings in patients with paraneoplastic antibodies necessitates a wide screening approach using immunohistochemistry rather than looking for a few specific antibodies; positive results can then be confirmed with more specific tests, such as Western blotting. The paraneoplastic antibodies can be detected both in CSF and serum. However, a positive CSF test in the absence of detectable antibodies in the serum is only rarely reported.[18] Therefore, for diagnostic screening purposes, serum detection suffices.

► DIAGNOSTIC EVALUATION

Clinical syndromes are never pathognomonic for a paraneoplastic etiology, and a high index of clinical suspicion is important. Symptoms can be atypical, psychiatric, or even fluctuating. PNS should often be in the differential diagnosis of otherwise unexplained neurologic syndromes. Detection of a typical antineuronal autoantibody (see Table 30-4) is very helpful because it proves the paraneoplastic etiology. Once a paraneoplastic diagnosis has



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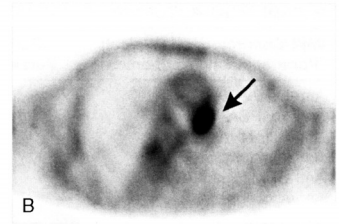

B

Figure 30-4. A patient with anti-Hu-associated encephalomyelitis had a negative CT of the chest. Four weeks after the CT, a total body FDG-PET was performed and demonstrated a hot spot in the left mediastinum. Histologic examination of a bronchoscopic biopsy showed SCLC.

been established or is suspected (in the absence of antibodies), rapid identification of the tumor becomes essential, but may be difficult. The type of antineuronal antibody will, in combination with the clinical syndrome, help direct the search for the underlying neoplasm (see Tables 30-2 and 30-3).

The workup for an underlying tumor starts with a detailed history including smoking habits, weight loss, night sweats, and fever. A thorough physical examination should include palpation for pathologic lymph nodes, rectal and pelvic examination, and palpation of breasts and testes. Routine laboratory tests including erythrocyte sedimentation rate should be carried out, and tumor markers can be added depending on the site under suspicion. The search for an underlying SCLC should further include chest CT. If the CT remains negative, FDG-PET is recommended (Fig. 30-4).[37] The workup for underlying breast cancer includes mammography (may be replaced by MRI) and cancer antigen (CA)-15-3 tumor marker. Gynecologic tumors are detected by a skilled pelvic examination, appropriate imaging studies, CA-125 tumor marker, and sometimes laparoscopy or laparotomy.[2] Testicular cancer is diagnosed on the basis of serum markers, physical and ultrasonography examination of the testes, and abdominal CT scan. Suspicion of prostate cancer leads to a determination of serum prostate-specific antigen levels and subsequent rectal examination. When the initial tumor workup is negative, a repeat workup is recommended at 3- to 6-month intervals for 2 to 3 years.

▶ NEUROPATHOLOGY AND PATHOGENESIS

Neuropathology

Denny-Brown, who described the first two patients with PSN, provided the earliest report of the pathologic changes in PNS.[97] The pathology demonstrated loss of dorsal root ganglion cells with heavy inflammatory infiltration in the dorsal root ganglia. In the Hu syndrome, the infiltrating cells have been further characterized as CD4+ T-helper cells and B-cells in the perivascular spaces and cytotoxic CD8+ T-cells in the interstitial spaces.[159] Further immunohistochemical analysis showed that a great number of T

cells express the cytotoxic protein TIA-1. By contrast, Fas, FasL, C9neo, and activated caspase-3 immunoreactivities were negative in pathologic areas. These data indicate that neuronal death in anti-Hu–associated PEM is cytotoxic and nonapoptotic.[160] To further elucidate the pathogenic role of the anti-Hu antibody, elution of Hu-specific IgG from the affected regions of the brain has been attempted.[159,161] Although Hu-specific IgG could be eluted from involved brain areas, an artifact could not be excluded. In addition, complement activation was lacking.[159,161]

In contrast, in anti-Yo– and anti-Tr–associated PCD there is complete loss of Purkinje cells, with minimal or no identifiable inflammatory changes.[18,162] Lack of inflammatory cells in these patients most likely reflects a final, burned-out stage of the immune disorder.[162] More remarkably, in some patients with paraneoplastic opsoclonus–myoclonus, autopsy revealed an entirely normal brain. Even the omnipause neurons that are responsible for opsoclonus were normal.[163]

Pathogenesis

The discovery of paraneoplastic antineuronal autoantibodies resulted in the general belief that these are immune-mediated disorders triggered by aberrant expression of "onconeural" antigens in the tumor. Support for this hypothesis comes from the fact that the target paraneoplastic antigens are expressed both in the tumor and in the affected parts of the nervous system. Furthermore, the tumors are usually small, heavily infiltrated with inflammatory cells, and have a better prognosis; spontaneous remission at the time of neurologic presentation has even been described.[164,165] In addition, the paraneoplastic antibodies are synthesized intrathecally and antigen-specific T cells have been demonstrated in the blood.[166–168] A pathogenic role could, however, only be proven for paraneoplastic autoantibodies directed against easily accessible antigens located at the cell surface. Examples of such antigens are the acetylcholine receptor (anti-AChR muscle type in myasthenia gravis and neuronal ganglionic type in autonomic neuropathy), anti-VGCC in LEMS, anti-VGKC in neuromyotonia, and anti-mGluR1 in PCD. Most paraneoplastic antigens are located in the cytoplasm (e.g., Yo) or nucleus (e.g., Hu and Ri) of the cell, and a pathogenic role for the respective antibodies could not be demonstrated.[169] In these disorders, indirect lines of evidence support the view that the cellular immune response against these antigens is responsible for the neurologic damage.[166,170] The paraneoplastic antibodies may, in these cases, be surrogate markers for T-lymphocyte activation.[21] Another unanswered question is why only a small proportion of patients with SCLC develop an "anti-Hu syndrome," whereas all SCLC express the Hu antigens. Development of the "anti-Hu syndrome" is not associated with human leukocyte antigen (HLA) alleles, rendering a genetic "autoimmune" disposition unlikely.[171] Careful examination of SCLC samples from anti-Hu–positive patients showed markedly increased major histocompatibility complex expression compared to tumor samples from seronegative patients.[48] Recent findings in LEMS also indicate that SCLC can trigger a strong autoimmune reaction in the absence of the proper HLA genotype.[172]

A totally different mechanism seems at work in PCD in Hodgkin disease because the target antigens of the associated anti-Tr and anti-mGluR1 autoantibodies are not expressed in the Hodgkin tumor tissue.[18] Dysregulation of the immune system in Hodgkin lymphoma and an etiologic role for (viral?) infections have been postulated in this disorder.

▶ TREATMENT AND PROGNOSIS

Treatment of PNS is directed at suppression of the autoimmune reaction and eradication of the underlying tumor (see Table 30-5). Despite the immunologic etiology of most PNS, the results of immunosuppressive treatment are disappointing.[41] Exceptions are the neurologic syndromes caused by functional interference of the paraneoplastic antibodies with the target receptor or channel. These include not only disorders of the peripheral nervous system (LEMS, myasthenia gravis, neuromyotonia, autonomic neuropathy), but also anti-mGluR1–associated PCD.[19] In paraneoplastic syndromes where the target antigen is cytoplasmic or nuclear, the nervous dysfunction is probably not caused by functional interference of antibodies with the antigen. Immunotherapy modalities that are recommended for disorders associated with antibodies against cell surface receptor or membrane antigens include plasma exchange, immunoadsorption (extraction of patient IgG over a protein A column), steroids, and IVIg. In disorders associated with intracellular target antigens and a strong cellular immune reaction, plasma exchange and immunoabsorption are not expected to give much benefit. In these cases, a trial of a treatment that modulates the activation and function of effector T cells makes more sense. Based on the literature, it is not possible to recommend either steroids with or without cyclophosphamide, IVIg, or any other treatment.

The first goal of antitumor treatment is control of the tumor. In addition, successful treatment of the tumor stops the paraneoplastic neurologic deterioration and leaves the patients, on average, in a better condition.[104] In severely debilitated patients, for example, the elderly and bedridden, treatment of an underlying tumor is often withheld because of the very small chance of clinically relevant neurologic improvement.

► **TABLE 30-5.** TREATMENT SUMMARY OF PARANEOPLASTIC NEUROLOGIC SYNDROMES

Syndrome	Antibody	Immunotherapy	Antitumor Treatment	Comments
PCD	Anti-Yo	No established effect	No effect on neurologic outcome	
	Anti-Tr	May improve	May improve	PCD associated with Hodgkin lymphoma may also improve spontaneously
	Anti-mGluR1	May improve	No data	
PEM	Anti-Hu	No established effect	Stabilizes the patient in better condition	In limbic encephalitis, partial improvement may occur spontaneously or during treatment
POM	Anti-Ri (adults)	No established effect	Partial neurologic recovery	Thiamine, baclofen, and clonazepam may be effective
	No antibody (children)	ACTH, steroids	Partial neurologic recovery	
SPS	Antiamphiphysin	IVIg, steroids, PE, pulse cyclophosphamide/steroids effective	No data	Responds to baclofen, diazepam, valproate, vigabatrine, and carbamazepine; Painful spasms may require opioids
CAR	Antirecoverin	No established effect	No established effect	
LEMS	Anti-VGCC	IVIg, steroids, PE effective	Improves neurologic condition	3,4-Diaminopyridine effective symptomatic treatment; immunotherapy less effective than in MG

CAR = cancer-associated retinopathy; IVIg = intravenous immunoglobulins; LEMS = Lambert-Eaton myasthenic syndrome; PCD = paraneoplastic cerebellar degeneration; PE = plasma exchange; PEM = paraneoplastic encephalomyelitis; POM = paraneoplastic opsoclonus–myoclonus; SPS = stiff-person syndrome; VGCC = voltage-gated calcium channels.

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CHAPTER 3

PARANEOPLASTIC CEREBELLAR DEGENERATION ASSOCIATED WITH ANTINEURONAL ANTIBODIES: ANALYSIS OF 50 PATIENTS

Satareh Shams'ili, Joost Grefkens, Bertie de Leeuw,
Martin van den Bent, Herbert Hooijkaas, Bronno van der Holt,
Charles Vecht and Peter Sillevis Smitt

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Summary

Paraneoplastic cerebellar degeneration (PCD) is a heterogeneous group of disorders characterized by subacute cerebellar ataxia, specific tumour types and (often) associated antineuronal antibodies. Nine specific antineuronal antibodies are associated with PCD. We examined the relative frequency of the antineuronal antibodies associated with PCD and compared the neurological symptoms and signs, associated tumours, disability and survival between groups of PCD with different antibodies. Also, we attempted to identify patient-, tumour- and treatment-related characteristics associated with functional outcome and survival. In a 12-year period, we examined >5000 samples for the presence of antineuronal antibodies. A total of 137 patients were identified with a paraneoplastic neurological syndrome and high titre (⩾400) antineuronal antibodies. Fifty (36%) of these patients had antibody-associated PCD, including 19 anti-Yo, 16 anti-Hu, seven anti-Tr, six anti-Ri and two anti-mGluR1. Because of the low number, the anti-mGluR1 patients were excluded from the statistical analysis. While 100% of patients with anti-Yo, anti-Tr and anti-mGluR1 antibodies suffered PCD, 86% of anti-Ri and only 18% of anti-Hu patients had PCD. All patients presented with subacute cerebellar ataxia progressive over weeks to months and stabilized within 6 months. The majority of patients in all antibody groups had both truncal and appendicular ataxia. The frequency of nystagmus and dysarthria was lower in anti-Ri patients (33 and 0%). Later in the course of the disease, involvement of non-cerebellar structures occurred most frequently in anti-Hu patients (94%). In 42 patients (84%), a tumour was detected. The most commonly associated tumours were gynaecological and breast cancer (anti-Yo and anti-Ri), lung cancer (anti-Hu) and Hodgkin's lymphoma (anti-Tr and anti-mGluR1). In one anti-Hu patient, a suspect lung lesion on CT scan disappeared while the PCD evolved. Seven patients improved by at least 1 point on the Rankin scale, while 16 remained stable and 27 deteriorated. All seven patients that improved received antitumour treatment for their underlying cancer, resulting in complete remission. The functional outcome was best in the anti-Ri patients, with three out of six improving neurologically and five were able to walk at the time of last follow-up or death. Only four out of 19 anti-Yo and four out of 16 anti-Hu patients remained ambulatory. Also, survival from time of diagnosis was significantly worse in the anti-Yo (median 13 months) and anti-Hu (median 7 months) patients compared with anti-Tr (median >113 months) and anti-Ri (median >69 months). Patients receiving antitumour treatment (with or without immunosuppressive therapy) lived significantly longer [hazard ratio (HR) 0.3; 95% confidence interval (CI) 0.1–0.6; $P = 0.004$]. Patients ⩾60 years old lived somewhat shorter from time of diagnosis, although statistically not significant (HR 2.9; CI 1.0–8.5; $P = 0.06$).

Keywords: autoantibodies; paraneoplastic; cerebellar ataxia; cancer; immunotherapy

Abbreviations: Ab = antibody; CR = complete remission; OS = overall survival; PCD = paraneoplastic cerebellar degeneration; PEM/SN = paraneoplastic encephalomyelitis/sensory neuropathy; PNS = paraneoplastic neurological syndrome; RS = Rankin scale; SCLC = small cell lung carcinoma

Introduction

Subacute cerebellar ataxia in a patient with a known cancer is often due to metastatic invasion or other complications of the cancer, such as infection, coagulopathy, metabolic and nutritional deficits or side effects of treatment (Henson and Urich, 1982). When tumour- and treatment-related causes have been excluded, the patient is considered to suffer from paraneoplastic cerebellar degeneration (PCD). In adult patients who are not known to have a malignancy, subacute cerebellar ataxia may cause a diagnostic challenge, as PCD can precede the presentation of a neoplasm by several months to years (Posner, 1995). In these patients, the detection of high titre antineuronal autoantibodies directed against onconeural antigens establishes the diagnosis of PCD and directs the search towards an underlying neoplasm (Table 1).

Brouwer first described PCD in 1919 (Brouwer, 1919), but the association of cerebellar ataxia and cancer was not recognized until 1938 (Brouwer and Biemond, 1938). Ovarian, lung and breast cancer and Hodgkin's lymphoma are the neoplasms most commonly associated with PCD (Henson and Urich, 1982). The pathology of PCD is characterized by severe loss of cerebellar Purkinje cells and the presence of inflammatory infiltrates in affected areas of the nervous system (Henson and Urich, 1982). Trotter *et al*. (1976) first described autoantibodies reactive with cerebellar Purkinje cells in the serum of a patient with Hodgkin's lymphoma and PCD. Since then, the search for paraneoplastic antineuronal antibodies (Abs) associated with PCD has resulted in the identification of many paraneoplastic Abs and the subsequent cloning of their onconeural target antigens (Table 1). PCD represents a heterogeneous group of related disorders that differ in their clinical features, prognosis, associated neoplasms and type of paraneoplastic Abs (Posner, 1995).

We reviewed 137 subjects with a definite, Ab-associated, paraneoplastic neurological syndrome (PNS) in order to determine the relative frequency of PCD. We subsequently studied the 50 identified PCD patients to compare neurological signs and symptoms, associated tumours, disability and survival between groups of patients with different Abs. In addition, we attempt to identify patient-, tumour- and treatment-related characteristics associated with neurological disability and survival.

Methods

Immunological studies

In a 12-year period (1989–2001), we examined serum and CSF samples from ~5000 patients. Physicians who suspected that their patients suffered from PNS had submitted the patient samples to our laboratory for determination of paraneoplastic antineuronal Abs. During the entire study period, all samples were screened on rat frozen cerebellar sections using indirect immunofluorescence (Moll *et al*., 1993). All positive sera were confirmed by western blotting, using rat cerebellar extract (Moll *et al*., 1993; Honnorat *et al*., 1998) and purified recombinant HuD, CDR62, amphiphysin and NOVA-1 antigens (Fathallah-Shaykh *et al*., 1991; Buckanovich *et al*., 1993; De Camilli *et al*., 1993; Manley *et al*., 1995). Anti-Tr was diagnosed by strict immunohistochemical criteria when Purkinje cell cytoplasmic staining was combined with a characteristic punctuated staining of Purkinje cell dendrites (Graus *et al*., 1998). Anti-mGluR1 was confirmed by reactivity with mGluR1-expressing Chinese hamster ovary (CHO) cells (Sillevis Smitt *et al*., 2000). Titration was performed by indirect immunofluorescence, and titres were reported as the reciprocal of the highest dilution yielding clearly positive staining. Only sera with specific staining patterns at titres $\geqslant$400 combined with reactivity with purified onconeural fusion protein were considered in this study. Patients were considered affected by PCD when they presented with cerebellar ataxia not attributable to metastases, infection, metabolic, hereditary, toxic or iatrogenic causes combined with the detection of high titre ($\geqslant$400) antineuronal Ab.

Subjects

We have identified a total of 137 patients with high titre antineuronal Abs. Fifty (36%) of these patients had PCD, including 19 anti-Yo, 16 anti-Hu, seven anti-Tr, six anti-Ri and two anti-mGluR1 (Table 2). While 100% of patients with anti-Yo, anti-Tr or anti-mGluR1 Abs suffered PCD, 86% of anti-Ri and only 18% of anti-Hu patients had PCD. None of the patients with anti-amphiphysin or anti-CV2 Abs in our series presented with PCD. Fourteen of the 50 PCD patients were seen at the Erasmus University Medical Centre Rotterdam, and the hospital charts of these patients were reviewed. Samples from the other 36 patients had been submitted by physicians, mostly from The Netherlands and Belgium, and clinical information was obtained from review of the case records sent to us by their outside medical specialists. Follow-up information was obtained from the patients' general practitioners.

Patient information was reviewed for neurological signs and symptoms at presentation and after stabilization of the syndrome. CNS dysfunction was classified as cerebellar degeneration, limbic encephalitis, brainstem encephalitis or myelitis. Cerebellar signs and symptoms were classified further as ataxia (predominantly truncal, appendicular or both), nystagmus and dysarthria. Signs and symptoms of peripheral nervous system dysfunction were classified as peripheral neuropathy (sensory, mixed somatic, autonomic or motor neuronopathy), focal neuropathy or radiculopathy. Fifty patients with predominantly cerebellar symptoms and signs at presentation were identified (Table 2).

The neurological disability was scored using a modified Rankin scale (RS) (Graus *et al*., 1992; Keime-Guibert *et al*., 1999) at the time of diagnosis (first positive antineuronal Ab result), before onset of treatment and at last follow-up. On the modified RS, a score of 0 represents an asymptomatic patient;

Table 1 *Paraneoplastic antibodies in PCD*

Antibody	Clinical syndromes	Immunohistochemistry	Western blot cerebellar extract	Gene	Associated cancer	Reference
Anti-Yo	Cerebellar ataxia	Cytoplasm of Purkinje cells and large brainstem neurons	34, 52 and 62 kDa	cdr34, cdr62-1, cdr62-2	Ovarian, breast	Fathallah-Shaykh *et al.* (1991); Peterson *et al.* (1992)
Anti-Hu	Cerebellar ataxia, PEM/SN	Nuclei of all neurons, nucleolar sparing	35–40 kDa	HuD, HuC, Hel-N1	SCLC	Szabo *et al.* (1991); Dalmau *et al.* (1992)
Anti-Ri	Cerebellar ataxia, OM	Nuclei of all central neurons, with nucleolar sparing	55 and 80 kDa	NOVA-1, NOVA-2	Breast, gynaecological, SCLC	Luque *et al.* (1991); Buckanovich *et al.* (1993)
Anti-Tr	Cerebellar ataxia	Cytoplasm and dendrites of Purkinje cells	–	Unknown	Hodgkin's lymphoma	Graus *et al.* (1997, 1998)
Anti-VGCC	Cerebellar ataxia, LEMS	–	–	CACNA1A	SCLC (60%)	Mason *et al.* (1997)
Anti-Ma	Cerebellar ataxia, brainstem dysfunction	Nuclei and cytoplasm of neurons	37 and 40 kDa	Ma1-5	Many	Dalmau *et al.* (1999)
Anti-Ta/Ma2	Limbic encephalopathy, cerebellar ataxia	Nuclei and cytoplasm of neurons	40 kDa	Ma2	Testis	Voltz *et al.* (1999)
Anti-CRMP5/CV2	PEM/SN, cerebellar ataxia	Cytoplasm of oligodendrocytes	66 kDa	CRMP5	SCLC, thymoma, gynaecological	Honnorat *et al.* (1996, 1999)
Anti-mGluR1	Cerebellar ataxia	Cytoplasm of Purkinje cells and brush cells, climbing fibres	–	MGluR1	Hodgkin's lymphoma	Sillevis Smitt *et al.* (2000)

OM = opsoclonus/myoclonus; VGCC = voltage-gated calcium channels; LEMS = Lambert–Eaton myasthenic syndrome.

Table 2 *Main clinical syndromes at presentation and high titre (⩾400) paraneoplastic antineuronal autoantibodies detected over a 12-year period (1989–2001)*

Antibody	*n*	PCD (%)	PSN	PLE	PEM	POM	SPS
Anti-Hu	90	16 (18)	46	14	13	1	–
Anti-Yo	19	19 (100)	–	–	–	–	–
Anti-Tr	7	7 (100)	–	–	–	–	–
Anti-Ri	7	6 (86)	–	–	–	1	–
Anti-amphiphysin	7	–	4	1	1	–	1
Anti-CV2	5	–	3	1	1	–	–
Anti-mGluR1	2	2 (100)	–	–	–	–	–
Total	137	50 (37)	53	16	15	2	1

PSN = paraneoplastic sensory neuropathy; PLE = paraneoplastic limbic encephalitis; PEM = paraneoplastic encephalomyelitis; POM = paraneoplastic opsoclonus/myoclonus; SPS = stiff person syndrome.

1, symptoms that do not interfere with lifestyle; 2, symptoms that lead to some restriction of lifestyle but do not prevent totally independent existence; 3, symptoms that significantly interfere with lifestyle or prevent totally independent existence; 4, symptoms that clearly prevent independent existence, although the patient does not need constant attention; and 5, severe disability is present with total dependence requiring constant attention. A patient was considered neurologically improved or deteriorated if there was a change of at least 1 point in the RS score measured at the time of diagnosis (positive Ab test) or onset of the treatment compared with the RS score at the time of stabilization of the symptoms (Keime-Guibert *et al*., 2000).

Statistical analysis

Due to the low number, the two patients with anti-mGluR1-associated PCD were excluded from the statistical analysis, leaving 48 patients. End points included overall survival (OS) from date of diagnosis (anti-neuronal Ab) and functional outcome at stabilization. OS was calculated until the date of death from any cause. Patients still alive at the date of last contact were then censored. OS was estimated by the Kaplan–Meier method, and Kaplan–Meier curves of the four anti-neuronal Ab groups were compared using the log-rank test.

The following variables were included in the analysis of prognostic factors: type of anti-neuronal Ab, age at diagnosis (<59 versus ⩾60 years), presence of tumour, RS score at diagnosis (0–3 versus 4–5) and treatment (no treatment versus immunotherapy only versus antitumour treatment with or without immunotherapy).

Patient characteristics of the four antineural Ab groups were compared using Fisher's exact test in the case of discrete variables or the Kruskal–Wallis test in the case of continuous variables.

Univariate Cox regression analysis was used to detect differences in OS between subgroups. Univariate logistic regression was used to test for a difference in functional outcome between subgroups.

We used Spearman's rank correlation to test whether there was an association between RS score at diagnosis and the time between onset of neurological symptoms and the date of diagnosis.

All P values are two-sided, and a significance level $\alpha = 0.05$ was used. All statistical analyses were performed using Stata software (StataCorp. 2001, Stata Statistical Software: Release 7.0, Stata Corporation, College Station, TX) and GraphPad Prism version 3.0 software (GraphPad Software, Inc., San Diego, CA, 1999).

Results

Patient characteristics

Patient characteristics are summarized in Table 3. All patients presented with a subacute cerebellar syndrome that evolved over weeks to months and stabilized within 6 months. The patients in the anti-Tr group tended to be younger than those in the other groups, reflecting the different age distribution of the associated tumours, in particular Hodgkin's disease. However, this was not statistically significant ($P = 0.4$). The median interval between the onset of symptoms and definite diagnosis of PCD (defined by detection of specific Ab) varied slightly between 3.5 months (anti-Yo) and 6.0 months (anti-Hu) ($P = 0.9$). At the time of diagnosis of PCD, the majority of patients in all Ab groups suffered both truncal and appendicular ataxia. The frequency of nystagmus and dysarthria was lower in anti-Ri patients (33 and 0%) than in the anti-Yo (68 and 84%), anti-Hu (69 and 50%) and anti-Tr (86 and 86%) patients. In all patients, the predominant neurological syndrome at the time of PCD diagnosis was cerebellar ataxia. Many developed additional, non-cerebellar symptoms or signs (Table 3). Involvement of non-cerebellar structures occurred most frequently in anti-Hu patients, indicating that PCD associated with anti-Hu Abs represents a more diffuse paraneoplastic encephalomyelitis. Despite these differences, the RS score at the time of diagnosis did not differ significantly between the patient groups ($P = 0.6$).

Associated tumours

The associated tumours in the five patient groups are summarized in Table 4. A tumour was diagnosed in 42 of

Table 3 *Clinical characteristics of 48 PCD patients*

	Anti-Yo	Anti-Hu	Anti-Tr	Anti-Ri	Anti-mGluR1
No. of patients	19	16	7	6	2
F/M	19/0	4/12	1/6	5/1	2/–
Age at diagnosis (median, range) (years)	64 (37–79)	67 (51–79)	54 (29–73)	65 (52–76)	19, 50
Additional neurological symptoms:					
Peripheral neuropathy	–	8	–	–	–
Limbic encephalitis	–	4	1	1	1
Brainstem encephalitis	6	10	–	3	–
Opsoclonus/myoclonus	–	1	–	4	–
Myelitis	–	1	–	1	–
Isolated PCD	13	1	6	2	1
Median delay between symptoms and diagnosis (range)	3.5 (0.3–52.8)	6.0 (0.9–18.4)	4.8 (1.2–13.0)	5.2 (0.8–13.3)	0.5 (0.5–12)
No. of patients with tumour	15	14	6	5	2
PCD before tumour diagnosis	5	13	6	2	–
Median interval PCD to tumour diagnosis (range)	3.5 (0.3–52.8)	6.0 (0.9–18.4)	4.8 (1.2–13.0)	5.2 (0.8–13.3)	–
Median survival (months)	13	7	>113	>69	24
Cause of death*					
Neurological	8	6	1	1	–
Oncological	4	2	–	–	–
Unknown/unrelated	–	2	–	–	1

*Death was scored as oncological when the patient died from tumour progression and as neurological when the paraneoplastic process contributed directly to death (e.g. in brainstem encephalitis) or when the neurological disability was judged to be a major contributing factor to death (e.g. pneumonia in a bedridden patient).

Table 4 *Associated tumours in 50 PCD patients*

Antibody	*n*	Lung	Gynaecological	Breast	Hodgkin's	Other	No tumour
Anti-Yo	19	–	9	3	–	3	4
Anti-Hu	16	14	–	–	–	–	2
Anti-Tr	7	–	–	–	6	–	1
Anti-Ri	6	–	1	3	–	1	1
Anti-mGluR1	2	–	–	–	2	–	–
Total	50	14	10	6	8	4	8

50 PCD patients (84%). In 26 (62%) of these tumour patients, the PCD preceded the diagnosis of the tumour. In 10 of the 15 anti-Yo patients (67%) with a tumour, the PCD presented in patients already known to have a cancer. Nine of these patients had reached complete remission (CR) following initial antitumor treatment. In five, the PCD was shortly followed by tumour recurrence; in three, the tumour recurred prior to PCD; and two remained free of recurrent tumour. One patient died 3 months after onset of PCD, 2.5 years after reaching CR for ovarian cancer. At autopsy, no recurrent tumour was found and neuropathological examination demonstrated complete loss of Purkinje cells. The second patient developed PCD 22 months after reaching CR for ovarian cancer. At last follow-up, 2 years after onset of PCD, regular gynaecological examinations had not yet revealed a recurrence.

In eight patients, no tumour was demonstrated. Four anti-Yo patients had no detected tumour. Two of these patients were still alive at the time of last follow-up, 44 and 58 months from onset of symptoms. Of the two deceased patients, one had mildly elevated CA125 and weight loss. She probably had an underlying ovarian cancer that could not be demonstrated, and died 32 months after onset of symptoms. The second deceased patient was in a poor condition; the family declined an extensive work-up and she died 9 months after onset of PCD. Due to the limited work-up, an underlying malignancy has not been excluded.

Of the two anti-Hu patients without demonstrable tumour, one had a suspect adrenal lesion possibly representing an adrenal small cell lung carcinoma (SCLC) metastasis. This diagnosis was not pursued further because of the patient's poor clinical condition, and she died 10 months after the diagnosis of PCD in a nursing home. The other patient is a 58-year-old man with a long history of cigarette smoking who presented with a mainly appendicular ataxia. He subsequently developed a pancerebellar syndrome and brainstem signs. Anti-Hu Abs were tested positive 18 months after onset of symptoms. The patient remained ambulatory but could not

Table 5 *Neurological outcome in 50 PCD patients*

Functional status	Anti-Yo	Anti-Hu	Anti-Tr	Anti-Ri	Anti-mGluR1	All patients
No. of patients	19	16	7	6	2	50
At diagnosis						
RS score <3	11	9	6	4	1	31
RS score ⩾4	8	7	1	2	1	19
Evolution RS score						
Improved (⩾1 point)	1	1	1	3	1	7
Stable	4	7	3	1	1	16
Deterioration (⩾1 point)	14	8	3	2	–	27
At plateau						
RS score <3	4	4	3	5	1	17
RS score ⩾4	15	12	4	1	1	33

Table 6 *Neurological outcome by treatment in 50 PCD patients*

Treatment/outcome	Anti-Yo	Anti-Hu	Anti-Tr	Anti-Ri	Ant-mGluR1	All patients
No. of patients	19	16	7	6	2	50
Patients with tumour	15	14	6	5	2	42
Antitumour treatment	5	1	3	1	–	10
Improved/stable	–	1	2	–	–	3
Immunosuppression	4	5	–	3	2	14
Improved/stable	2	2	–	2	1	7
Both treatments	6	2	3	2	–	13
Improved/stable	2	2	2	2	–	8
No treatment	4	7	1	–	–	12
Improved/stable	2	4	–	–	–	6

Patients are considered improved or stable when the RS score from time of diagnosis of PCD did not deteriorate.

live independently. A CT scan demonstrated a suspect lesion in the left upper lobe. The lesion disappeared spontaneously and, at the time of last follow-up, 68 months after onset of the ataxia, the patient was alive without signs of cancer.

In one anti-Tr and one anti-Ri patient, no tumour was found. The anti-Tr patient died 10 months after PCD onset. At autopsy, no tumour was detected. The anti-Ri patient has remained free of cancer during 4.5 years of follow-up.

Neurological outcome

The neurological outcome is presented in Table 5. In general, at the time of neurological diagnosis, 63% of patients were ambulatory (RS score <3). When PCD had reached its plateau phase, only 34% were still able to walk. The functional outcome was significantly different between the four patient groups ($P = 0.04$). Outcome was worst in the anti-Yo patients (79% bedridden) followed by anti-Hu (75%), anti-Tr (57%) and anti-mGluR1 (50%) patients. The prognosis in the anti-Ri patients was much better, and 83% retained their ability to walk. Using logistic regression, age ⩾60 years [odds ratio (OR) 5.4; 95% confidence interval (CI) 1.4–21.2; $P = 0.02$] and a higher RS score at diagnosis (OR 3.8; 95% CI 0.9–16.1; $P = 0.07$) predicted worse functional outcome (RS 4–5). Neither immunotherapy (OR 0.4; 95% CI 0.1–2.8; $P = 0.4$) nor antitumour treatment (OR 0.3; 95% CI 0.1–2.0; $P = 0.3$) significantly affected functional outcome (Table 6). Seven patients (14%) improved neurologically from the time of diagnosis or start of treatment: three anti-Ri patients and one each with anti-Yo, anti-Hu, anti-Tr and anti-mGluR1 Abs (Table 7). Remarkably, all patients who improved neurologically received antitumour treatment, and CR was achieved in all. Patient 2 (Table 7) developed PCD shortly after diagnosis of a lymph node metastasis in the groin, probably from an ovarian cancer. At presentation, her RS score was 3 (ambulatory) but she deteriorated despite intravenous immunoglobulin G (IVIg) treatment and became bedridden (RS score = 4) prior to transfer to our hospital. After obtaining informed consent, she was treated with four cycles of rituximab (375 mg/m^2). After the first cycle, she regained her ability to walk (RS score = 3) and remained stable for >1 year. Rituximab (Roche Ltd, Basel, Switzerland) is a chimeric mouse–human anti-CD20 monoclonal Ab effectively used in the treatment of B-cell lymphomas (Maloney *et al*., 1994, 1997). Rituximab has also been effective in a variety of Ab-associated autoimmune disorders (Arzoo *et al*., 2002). In patient 2, the rituximab treatment eliminated circulating CD19$^+$ B cells but did not result in lower anti-Yo titres in serum (Table 7) or CSF. In the patients with anti-Tr and anti-mGluR1, the Abs had disappeared from the serum

Table 7 *Characteristics of seven PCD patients with improvement of functional status by at least 1 point on the modified Rankin scale*

No.	Antibody	Age	Tumour	Antitumour treatment	Immunosuppression	RSD	RSE	STD	STE
1	Anti-Hu	66	SCLC	CR	None	4	1	>1600	NA
2	Anti-Yo	47	Adenocarcinoma	CR	IVIg, rituximab	3*	3	6400	12 800
3	Anti-Tr	31	Hodgkin	CR	None	3	0	1600	Neg
4	Anti-Ri	58	Breast cancer	CR	PE, steroids, azathioprine	3	2	25 600	800
5	Anti-Ri	56	Tuba cancer	CR	Steroids	4	1	12 800	102 400
6	Anti-Ri	53	Breast cancer	CR	IVIg, steroids	4	2	3200	NA
7	Anti-mGluR1	19	Hodgkin	CR	IVIg, steroids, PE	3	0	3200	Neg

RSD = RS score at diagnosis; RSE = RS score at last follow-up; STD = serum antibody titre at diagnosis (IgG); STE = serum antibody titre after clinical improvement (IgG); NA = not available; Neg = negative; PE = plasma exchange. *This patient was diagnosed with PCD while still ambulatory (RSD = 3). She subsequently became bedridden (RS score = 4) despite treatment with IVIg. Following treatment with rituximab, she regained her ability to walk (RS score = 3) for > 1 year (see Results).

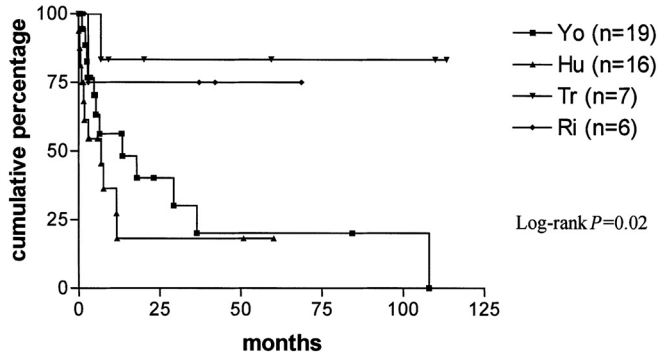

Fig. 1 Kaplan–Meier survival curves of 48 PCD patients with associated anti-Yo, anti-Hu, anti-Tr or anti-Ri antibodies.

at the time of clinical improvement (Table 7). In one of the anti-Ri patients, clinical improvement was paralleled by a decline in Ab titre, while high titres remained in the second anti-Ri patient despite neurological improvement. No follow-up serum was available from the third anti-Ri and the anti-Hu patients who had improved clinically.

Survival

The median survival of anti-Hu patients from time of diagnosis was 7 months, compared with 13 months in anti-Yo patients (Fig. 1). This difference in survival was statistically not significant [hazard ratio (HR) 1.7; 95% CI 0.7–3.9; P = 0.2]. Anti-Tr patients (median >113 months) lived significantly longer from time of first symptoms than anti-Yo (HR 0.12; 95% CI 0.02–0.97; P = 0.05) and anti-Hu patients (HR 0.13; 95% CI 0.02–1.00; P = 0.05). Anti-Ri patients (median >69 months) also lived longer than anti-Yo and anti-Hu patients, but the differences were not significant.

In patients ⩾60 years, there was a non-significant trend towards shorter survival from time of diagnosis (HR 2.9; 95% CI 1.0–8.5; P = 0.06). Patients receiving antitumour treatment (with or without immunosuppressive therapy) lived significantly longer (HR 0.3; 95% CI 0.1–0.6; P = 0.004). However, after adjustment for Ab group, there was no statistically significant treatment effect.

Discussion

We found that 36% of 137 patients with a definite PNS presented with subacute cerebellar ataxia, indicating that PCD is a common presentation of Ab-associated PNS. While anti-Hu was the most frequent paraneoplastic Ab detected in our study (66% of 137 patients), only 18% presented with PCD. In contrast, 100% of anti-Yo, anti-Tr and anti-mGluR1, and 86% of anti-Ri patients presented with PCD. In PCD patients, anti-Yo (38%) was detected most frequently, followed by anti-Hu (32%), anti-Tr (14%) and anti-Ri (12%) Abs. During the course of the disease, additional

non-cerebellar symptoms occurred most frequently in the anti-Hu patients (94%), indicating that PCD associated with anti-Hu Abs is part of a more widespread paraneoplastic encephalomyelitis/sensory neuronopathy (PEM/SN) (Dalmau *et al*., 1992; Graus *et al*., 2001; Sillevis Smitt *et al*., 2002).

The median age of the anti-Hu, anti-Yo and anti-Ri patients was 64–67 years and of the anti-Tr patients was 54 years. The latter median age is remarkable because all anti-Tr patients had associated Hodgkin's lymphoma. The age distribution of Hodgkin's lymphoma is bimodal, with an early peak in young adults and a second peak after age 50 (Gutensohn and Cole, 1980). Most anti-Tr and one of the anti-mGluR1 PCD patients belong to the second age peak, which may reflect a different mechanism in the pathogenesis of the Hodgkin's lymphoma between the two age groups.

Survival varied significantly between PCD patients with different Abs. The median survival from time of diagnosis in the anti-Hu patients was 7 and in anti-Yo patients 13 months, confirming the grim prognosis (Rojas *et al*., 2000; Graus *et al*., 2001; Sillevis Smitt *et al*., 2002). In contrast, the median survival in the anti-Tr (>117 months) and anti-Ri (>69 months) was not reached. Other factors predicting longer survival were administration of antitumour treatment and younger age.

In our study, 67% of the deceased anti-Yo patients died of a neurological cause, confirming two earlier reports (Hammack *et al*., 1990; Peterson *et al*., 1992). In contrast, Rojas *et al*. (2000) described death by a neurological cause in only 29% of anti-Yo patients, the majority dying from tumour progression. This difference is not explained by the associated malignancies. The most common associated tumours in anti-Yo patients were gynaecological (Hammack *et al*., 1990; Peterson *et al*., 1992; Rojas *et al*., 2000), in anti-Hu patients lung cancer (Dalmau *et al*., 1992; Graus *et al*., 2001; Sillevis Smitt *et al*., 2002), in anti-Tr and anti-mGluR1 patients exclusively Hodgkin's lymphoma (Graus *et al*., 1997), and in anti-Ri patients breast cancer (Luque *et al*., 1991). No tumour was detected in eight patients, while in one anti-Hu patient a radiologically suspect lung lesion disappeared spontaneously during the course of PCD. Remissions of radiologically suspect lung lesions and histologically confirmed SCLC during anti-Hu-associated PEM/SN have been reported previously (Darnell and DeAngelis, 1993; Zaheer *et al*., 1993) and support an important role for HuD-specific cytotoxic T lymphocytes (Benyahia *et al*., 1999; Plonquet *et al*., 2002).

In accordance with previous studies, 75% of anti-Hu patients became chair bound (Graus *et al*., 2001; Sillevis Smitt *et al*., 2002). Although symptoms were limited to the cerebellum in most, only 21% of the anti-Yo patients remained ambulatory, confirming previous reports of the grim neurological prognosis (Hammack *et al*., 1990; Peterson *et al*., 1992; Rojas *et al*., 2000). Neurological disability in anti-Tr and anti-mGluR1 was less severe, and 43 and 50% remained ambulatory, respectively. Anti-Ri PCD patients had the best neurological prognosis; 83% remained ambulatory and three out of six patients improved neurologically, confirming previous case reports (Dropcho *et al*., 1993; Jongen *et al*., 1998).

We studied factors which were involved in the neurological disability. We found that age $\geqslant$60 years and a higher RS score at diagnosis predicted worse functional outcome, while neither immunosuppression nor antitumour treatment had a significant effect. Larger studies have demonstrated better neurological outcome in patients receiving antitumour treatment for anti-Hu associated PEM/SN (Graus *et al*., 2001), while no such effect was demonstrated for anti-Yo-related PCD (Rojas *et al*., 2000). However, of the seven patients (14%) who improved neurologically, all had reached a CR of the tumour following antitumour treatment, and four had been treated additionally with some form of immunotherapy. One patient with anti-Yo-associated PCD regained her ability to walk following rituximab (anti-CD20) treatment. CD20 is a transmembrane surface antigen expressed only by B-cell precursors and mature B cells, and appears to play an important functional role in B-cell activation, proliferation and differentiation (Tedder and Engel, 1994). Rituximab is very effective in the treatment of B-cell lymphomas (Maloney *et al*., 1994, 1997) and it is used increasingly in the treatment of Ab-mediated autoimmune disorders including paraneoplastic pemphigus (Heizmann *et al*., 2001), myasthenia gravis (Zaja *et al*., 2000) and polyneuropathy associated with IgM Abs (Levine and Pestronk, 1999). Rituximab treatment results in long-lasting peripheral blood B-cell depletion, and we hypothesized that administration of rituximab would result in elimination of pre-B cells, preventing formation of new Ab-secreting cells and reducing anti-Yo titres not only in serum but also in CSF. Despite complete depletion of circulating $CD19^+$ B cells in our patient, her CSF and serum anti-Yo IgG titres remained high. Slight neurological fluctuations are common in anti-Yo-associated PCD, but a significant improvement rarely if ever occurs in this aggressive disorder (Peterson *et al*., 1992; Posner, 1995; Rojas *et al*., 2000), suggesting some role for rituximab in the neurological improvement in our patient. Although we do not fully understand the mechanism, rituximab treatment currently is under further evaluation. In three patients, the neurological improvement was paralleled by a decrease in Ab titre (one each with anti-Ri, anti-Tr and anti-mGluR1 Abs). In the anti-mGluR1 patient, the clinical improvement may have resulted directly from the disappearance of anti-mGluR1 from the serum because these autoantibodies have been shown to block the mGluR1 receptor *in vivo* (Sillevis Smitt *et al*., 2000). A pathogenic role for anti-Yo and anti-Hu Abs could never be proven in animal models (Graus *et al*., 1991; Sillevis Smitt *et al*., 1995), and demonstration of antigen-specific cytotoxic T lymphocytes in blood, CSF and target tissue (Benyahia *et al*., 1999; Albert *et al*., 2000; Plonquet *et al*., 2002) has shifted attention to a pathogenic role for the cellular immune response. Conventional immunosuppression is not effective in anti-Hu- and anti-Yo-associated PCD, and new treatment

modalities directed at a rapid destruction of the immune attack on the nervous system are warranted. Aggressive autoimmune disorders, including multiple sclerosis, are increasingly treated with haematopoietic stem cell transplantation following myelosuppressive conditioning (Burt *et al*., 1998; Fassas *et al*., 2002). In selected patients with an aggressive course of PCD, autologous haematopoietic stem cell transplantation may be considered in a carefully controlled protocol. In patients with a more indolent course and in patients with anti-Ri Abs, antitumour treatment and a trial of conventional immunotherapy is recommended.

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CHAPTER 4

ANTI-TR ANTIBODIES AS MARKERS OF PARANEOPLASTIC CEREBELLAR DEGENERATION AND HODGKIN'S DISEASE

F. Bernal, PhD; S. Shams'ili, MD; I. Rojas, MD; R. Sanchez-Valle, MD; A. Saiz, MD; J. Dalmau, MD, PhD; J. Honnorat, MD, PhD; P. Sillevis Smitt, MD, PhD; and F. Graus, MD

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Abstract—*Background:* Preliminary studies suggested that anti-Tr antibodies identify patients with paraneoplastic cerebellar degeneration (PCD) and Hodgkin disease (HD). *Objective:* To extend the clinical–immunologic analysis to 28 patients with anti-Tr antibodies. *Methods:* Anti-Tr antibodies were detected by immunohistochemistry. A competitive inhibition assay was used to ascertain if anti-Tr antibodies of different sera identify common epitopes. Anti-Tr immunoglobulin G (IgG) subclass distribution was determined by immunohistochemistry using monoclonal antibodies against human IgG isotypes. Tr immunoreactivity was analyzed in tumor sections using biotinylated anti-Tr IgG. *Results:* Median age of the 28 patients was 61 years (range 14 to 75 years) and 22 were male. A cerebellar syndrome was present in 27 patients and a possible limbic encephalitis in one. HD was diagnosed in 25 patients. No tumor was found in three patients; the autopsy of one of them disclosed severe loss of Purkinje cells without inflammatory infiltrates. Anti-Tr antibodies spontaneously disappeared in all patients without tumor and in 10/10 patients after successful HD treatment. Anti-Tr antibodies were absent in the serum but positive in the CSF of two patients. All positive anti-Tr sera inhibited the immunoreactivity of biotinylated anti-Tr IgG. The predominant isotypes of anti-Tr were IgG1 and IgG3. Only 1 out of the 15 HD samples studied presented anti-Tr positivity that was localized in some Reed-Sternberg cells. *Conclusions:* This study confirms the strong association between anti-Tr antibodies and PCD associated with HD. Anti-Tr antibodies from different patients recognize similar epitopes. Unlike other antineuronal antibodies, anti-Tr antibodies can be detected in the CSF but not in the serum and may spontaneously disappear during the follow-up, and Tr immunoreactivity is usually lacking in the tumor.

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Hodgkin disease (HD) is the third most common cause of paraneoplastic cerebellar degeneration (PCD) after small cell lung carcinoma and adenocarcinomas of breast and ovary.[1] Initial studies described an anti-Purkinje cell antibody in patients with PCD and HD but the antibody was not characterized.[2-4] Later, we showed that the anti-Purkinje cell antibody found in five patients with PCD and HD produce a characteristic punctate immunoreactivity in the molecular layer of the rat cerebellum and named the antibody anti-Tr.[5] Laser confocal and immunoelectron microscopy localized the antigen in the shaft of the dendritic branchlets without specific concentration in the dendritic spines.[6] Attempts to characterize the anti-Tr antibodies by immunoblot or cloning of the antigen have been unsuccessful, suggesting that these antibodies recognize conformational epitopes. Therefore, the only way to identify the anti-Tr antibodies is by the immunohistochemical pattern.[5] In spite of this limitation, the guidelines proposed for the diagnosis[5] have been reproduced by other specialized laboratories on diagnosis of paraneoplastic neurologic disorders.[7,8]

In the current study, we extend the analysis to a series of 28 patients with anti-Tr antibodies and describe new clinical and immunologic features.

Patients and methods. We reviewed the clinical information of 28 patients whose serum or CSF, sent to our laboratories to detect antineuronal antibodies, showed anti-Tr immunoreactivity. The clinical information was obtained from forms filled out by the referring neurologists, telephone interviews, and review of the clinical records. Six patients were previously reported.[5,9]

anti-Tr antibody analysis. Anti-Tr antibodies were detected by immunohistochemistry (screening dilution serum 1:500; CSF undiluted) using an avidin-biotin technique on paraformaldehyde-fixed frozen sections of rat cerebellum as described in detail previously.[5] The diagnosis of anti-Tr immunoreactivity was made when, besides the staining of the Purkinje cell cytoplasm and proximal dendrites, there was a punctate staining in the dendritic branchlets in the molecular layer. In addition, neurons of the granular layer were negative, and those of dentate nucleus weakly positive (for a more detailed description see reference [5] and table 1

From the Service of Neurology and Institut d'Investigació Biomèdica August Pi i Sunyer (IDIBAPS) (Drs. Bernal, Sanchez-Valle, Saiz, and Graus), Hospital Clínic, University of Barcelona, Spain; Department of Neuro-Oncology (Drs. Shams'ili and Sillevis Smitt), Daniel den Hoed Cancer Center, University Hospital of Rotterdam, the Netherlands; Service of Neurology (Dr. Rojas), Ciutat Sanitaria Universitaria de Bellvitge, Hospitalet, Spain; Laboratory of Neuro-Oncology, Arkansas Cancer Research Center (Dr. Dalmau), Little Rock; and Service of Neurology B (Dr. Honnorat), Hôpital Neurologique, Lyon, France.

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Address correspondence and reprint requests to Dr. Francesc Graus, Service of Neurology, Hospital Clínic, Villarroel 170, E-08036 Barcelona, Spain; e-mail: graus@medicina.ub.es

of reference [8]). All samples were confirmed in a reference laboratory (F.G.)

To show if anti-Tr antibodies of different patients recognized similar epitopes, rat cerebellar sections were preincubated with undiluted anti-Tr-positive serum for 3 hours followed by a biotinylated immunoglobulin G (IgG) obtained from a positive anti-Tr serum, in 10% normal human serum, overnight at 4 °C, and the Vectastain Elite ABC complex (Vector Labs, Burlingame, CA) for 40 minutes. The reaction was developed with 0.05% diaminobenzidine and 0.01% hydrogen peroxide diluted in phosphate-buffered saline (PBS) with 0.5% Triton X-100. As controls, sections were preincubated with normal human and anti-Yo-positive serum.

To study the distribution of IgG subclasses and the presence of IgM anti-Tr antibodies, 17 sera, selected by high titer (>1:2,000) and enough volume available, were used. Frozen sections of rat cerebellum from animals previously perfused with cold PBS were fixed in cold acetone for 10 minutes. After inhibition of endogenous peroxidase with 0.3% H_2O_2 in PBS for 15 minutes, sections were sequentially incubated with anti-Tr positive serum (1:10 and 1:50 dilution) overnight at 4 °C and biotinylated mouse monoclonal antibodies to IgM (Southern Biotech, Birmingham, AL) or IgG subclasses (Sigma, St. Louis, MO) (dilutions: anti-IgM 1:800, anti-IgG1 1:100, anti IgG2 1:100, anti-IgG3 1:400, and anti-IgG4 1:200) for 30 minutes at room temperature. Sections were developed with the avidin-biotin immunoperoxidase technique described above. The punctate staining of the molecular layer of the cerebellum was used as criterion for specific anti-Tr reactivity. As controls, we included 6 normal human, 6 anti-Hu-, 10 anti-Yo-, and 1 anti-MAG-positive sera.

Tr immunoreactivity in HD tumor samples. To study if there was Tr immunoreactivity in samples of HD, frozen (2 samples) or paraffin (15 samples) sections of pathologic lymph nodes from 15 patients with Tr antibodies and HD were analyzed. Frozen sections were fixed in cold acetone or 4% paraformaldehyde. Paraffin sections were deparaffinized in xylene, rehydrated in alcohol, washed in tap water, and heated for 2 minutes in a pressure cooking oven in 0.1 M sodium citrate buffer (pH 6.0). After inhibition of endogenous peroxidase with 0.3% H_2O_2 in PBS, sections were sequentially incubated with undiluted normal human serum, biotinylated anti-Tr, or control IgG in 10% normal human serum overnight at 4 °C, and developed with the avidin-biotin immunoperoxidase technique described above. As positive control, we used a section of paraffin embedded rat cerebellum processed as a surgical pathology specimen.

Results. *Patients and clinical findings.* The clinical information of the 28 patients is summarized in the table. Twenty-two patients had a typical course characterized by subacute, irreversible severe cerebellar syndrome. The cerebellar syndrome was isolated except in two patients who also had encephalopathy and sensory neuropathy. Four patients had subacute pancerebellar syndrome that unlike that of the previous 22 patients showed a clear improvement. Two of them also presented a reversible encephalopathy and an optic neuritis. One patient developed mild gait ataxia 10 years after the cure of HD. The MR showed a moderate cerebellar atrophy. The ataxia remains stable and no tumor has been found after a follow-up of 4 years. Only one patient did not have cerebellar symptoms. He presented with a clinical picture suggestive of limbic encephalitis but results on MR were normal. Routine CSF analysis, available in 22 patients, was normal in 9. In the other 13 patients, there was a mild CSF pleocytosis with median white blood cells per dL of 50 (range 14 to 150). A trend was observed between remission of the neurologic syndrome and age. Among the seven patients aged 40 years or younger, 3 (43%) made a complete remission of the ataxia, whereas only one of the 21 patients (4.7%) older than 40 years made a partial remission.

A diagnosis of tumor was made in 25 patients; all had HD. The most common pathologic type was nodular sclerosis (14 patients) followed by mixed cellularity (4) and lymphocyte depletion (2). The HD could not be classified in five patients. The HD was diagnosed in a limited stage (I or II) in 19 (76%) of the patients. The neurologic syndrome antedated the diagnosis of the tumor in 20. In the five patients in whom the PCD was diagnosed after the HD, the paraneoplastic syndrome appeared during the treatment of the HD (1 patient), predicted the HD recurrence (1), and a third patient was lost to follow-up shortly after the diagnosis of the cerebellar syndrome when the HD was in remission. A fourth patient underwent hematopoietic stem-cell autotransplant in absence of tumor relapse and the PCD stabilized. The HD has not relapsed after 3 years of follow-up. Finally, the fifth patient was diagnosed with HD 10 years before the development of a mild, slowly progressive cerebellar syndrome.

Table *Clinical features of 28 patients with anti-Tr antibodies*

Characteristics	Values
Patients (M/F)	28 (22/6)
Age, y, median (range)	61 (14–75)
Main syndrome (n)	Paraneoplastic cerebellar degeneration* (23)
	Subacute cerebellar syndrome† (3)
	Chronic, mild cerebellar ataxia‡ (1)
	Limbic encephalitis (1)
Tumor type (n)	Hodgkin disease (25)
	No tumor (3)
Median (range) time, mo, between neurologic syndrome and tumor diagnosis (n)	3.5 (0–24) (n = 20)§
Clinical outcome	Complete remission of ataxia in three, partial in one; the rest of the patients stable, with bad functional status, or worse

* Defined by the temporal association with the tumor. Included four patients who had a complete or almost complete recovery.
† Tumor not found (see text). None of the patients improved.
‡ Patient diagnosed and cured of Hodgkin disease 10 years earlier.
§ Paraneoplastic cerebellar degeneration appeared after tumor diagnosis in five patients after 3, 12, 12, 102, and 120 months.

No tumor was detected in three patients with a typical picture of PCD: two have been followed for 8 months and 3 years, and the third patient died 10 months after symptom presentation with a severe subacute cerebellar ataxia. In this patient, a close clinical follow-up with repeated physical examinations, laboratory tests, CT scans of chest and abdomen, and a bone marrow biopsy did not reveal a malignancy. At autopsy, gross anatomic and microscopic examination showed no tumor. Neuropathologic analysis was performed on formalin-fixed, paraffin-embedded sections taken from selected areas of the cortex, hippocampus, cerebellum, and brainstem. The neuropathologic study disclosed a total loss of cerebellar Purkinje cells with mild Bergmann gliosis. The dentate nucleus and inferior olive demonstrated mild secondary neuronal cell loss and gliosis. Immunohistochemistry with markers against B (CD20) and T (CD3, CD8) cells, macrophage/microglia (CD68), and astrocytes (GFAP) disclosed only sporadic CD3 and CD68 positive inflammatory cells in the cerebellum.

Anti-Tr antibody characteristics and outcome. Serum anti-Tr titers were present in 26 of the 28 patients. The median titer (of 18 sera titrated) was 1:5,000 (range 1:500 to 1:80,000). All 13 evaluated CSF were anti-Tr positive (median titer 1:100; range 1:1 to 1:400). The two patients who had no serum anti-Tr antibodies (at the screening dilution of 1:500) showed Tr immunoreactivity in the paired CSF sample. Immunohistochemistry using lower serum dilutions was inconclusive owing to background staining. Serum anti-Tr antibodies became negative in the 10 serum samples obtained after successful treatment of the HD. By contrast, the anti-Tr titers remained unchanged during 2 years in a patient with HD detected in an enlarged axillary node that in retrospect was already present in the chest CT scan obtained at the onset of

the PCD 2 years earlier. In the patient who underwent an autologous stem-cell transplantation for the cerebellar syndrome by the time HD was in remission, the anti-Tr titers slowly decreased, but are still detectable in serum and CSF 2 years after the transplantation, whereas the cerebellar syndrome is unchanged and the HD continues in remission. Anti-Tr antibodies spontaneously disappeared in the three patients with severe cerebellar syndrome and no evidence of cancer.

Preincubation of the cerebellar rat sections with each of the anti-Tr-positive sera resulted in the abolition of the immunoreactivity observed by the biotinylated anti-Tr IgG, indicating that similar epitopes are recognized by all positive sera.

All tested anti-Tr antibodies had IgG1, IgG3, or both isotypes. Nine sera had IgG1 and IgG3 reactivity, with no remarkable differences in immunohistologic staining between the two subclasses. The presence of only one isotype was detected in eight sera, being 50% IgG1 or IgG3. The two sera with IgG2 or IgG4 reactivity were also IgG1 positive. No IgM positive immunoreactivity was detected. Anti-Hu-positive sera presented all four IgG subclasses, although not necessarily in all sera, and anti-Yo-positive sera showed only IgG1 reactivity. None of the anti-Hu, anti-Yo, or control sera showed the punctate pattern in the molecular layer that defined the Tr immunoreactivity.

Tr reactivity in HD tumor samples. Tr-positive cells were found in 1 of the 15 evaluated tumor samples. The positive immunoreactivity detected in paraffin-embedded sections was cytosolic, with a weak diffuse and more robust granular staining (figure). It was present in a few malignant cells, including some with morphology of Reed-Sternberg cells. The same results were obtained with biotinylated IgG from two positive anti-Tr sera. In addition, the reactivity was abolished if the tumor sample was preincubated with a positive nonbiotinylated anti-Tr serum.

Discussion. The current study confirms the tight association between the presence of anti-Tr antibodies and PCD associated with HD.[5] A tumor was not found in 3 (11%) patients but the follow-up is too short in one to rule out an underlying malignancy. The only patient who did not have ataxia developed a clinical picture that resembled limbic encephalitis. HD is an infrequent cause of limbic encephalitis, accounting for less than 10% of the cases reported.[10] In our patient, the normal MR findings did not allow us to make a more definitive diagnosis of limbic encephalitis, but up to 36% of patients with limbic encephalitis have a normal brain MR.[10]

The clinical course of PCD was usually subacute and irreversible as described in patients with anti-Yo or anti-Hu antibodies.[11,12] This feature agrees with the neuropathologic findings of severe Purkinje cell loss in the patient of this series without HD and previous autopsy reports of PCD associated with HD.[13,14] However, an important difference is that PCD remitted in 14% of our patients with anti-Tr antibodies, suggesting that cerebellar damage is not irreversible in all instances. Clinical improvement seems more likely in younger patients, a feature not previously emphasized.

In this study, PCD antedated the diagnosis of HD in 83% of the patients, in contrast to the 19% observed in another series of PCD and HD published before anti-Tr antibodies were chatacterized.[4] The reason for this discrepancy is unclear. It could be that anti-Tr antibodies associate with a specific subset of PCD that usually antedates the diagnosis of HD, whereas another subset, probably representing another immunologic specificity,[15] includes cases of cerebellar ataxia developing months or years after treatment. However, one of the patients in this series presented the cerebellar syndrome 10 years after the HD and all patients in a previous series who had anti-Purkinje cell antibodies (presumably anti-Tr) developed the PCD after the diagnosis of HD.[4]

In the current article, all positive sera inhibited the reactivity of a biotinylated anti-Tr IgG, indicating that the antibodies diagnosed by the common immunohistochemical pattern indeed recognize similar epitopes of presumably the same antigen. This immunocompetition assay, based in limited epitope usage, can be used as confirmatory test of anti-Tr reactivity. The occurrence of a limited epitope usage is frequent in paraneoplastic disorders and has been reported in antibodies against other onconeural antigens.[16,17]

Anti-Tr antibodies were found in the CSF but not in the serum of 2 (7%) patients. The failure to detect the anti-Tr antibodies in the serum, not reported with other onconeural antibodies, may be explained by the relatively low sensitivity of the immunohistochemistry technique. In fact, low titers of anti-Hu antibodies detected by immunoblot of recombinant HuD protein cannot be demonstrated by routine immunohistochemistry.[18] Another feature not reported with other paraneoplastic antibodies is that anti-Tr antibodies spontaneously disappeared in the three patients without HD and in all the patients who went into tumor remission. Although anti-Hu and anti-Yo titers may decrease after effective tumor treatment, they frequently remain positive over the ensuing years.[19-21] The prompt disappearance of anti-Tr antibodies after treatment of HD may reflect the high efficacy of antineoplastic treatment to cure HD (therefore eliminating the antigenic trigger). The spontaneous disappearance of anti-Tr antibodies in all three patients without HD is similar to that observed with antiganglioside antibodies in the Miller-Fisher syndrome[22] and suggests that in a few instances the origin of anti-Tr antibodies is not an underlying tumor but another (viral infection?) unknown cause. Alternatively, the Tr-mediated immune response could have eradicated the underlying HD, as described in a few patients with anti-Hu antibodies and remitting small cell lung carcinoma,[23] and later faded away.

The current study shows that IgG1 and IgG3 are the major subclasses of anti-Tr antibodies, a feature shared by many autoantibodies against organ and nonorgan-specific antigens,[24-26] except anti-Yo antibodies, which appear restricted to the IgG1 subclass, as previously described.[27] The predominance of the IgG1 and IgG3 subclasses in anti-Tr and other autoantibodies is believed to reflect a Th1-type response of $CD4^+$ T helper cells to the antigen.[28] However, the potential pathogenic role of this IgG isotype distribution is unclear considering the intracellular location of the target antigen.

The most probable origin of the immune response in paraneoplastic neurologic disorders associated with solid tumors is the expression of neuronal anti-

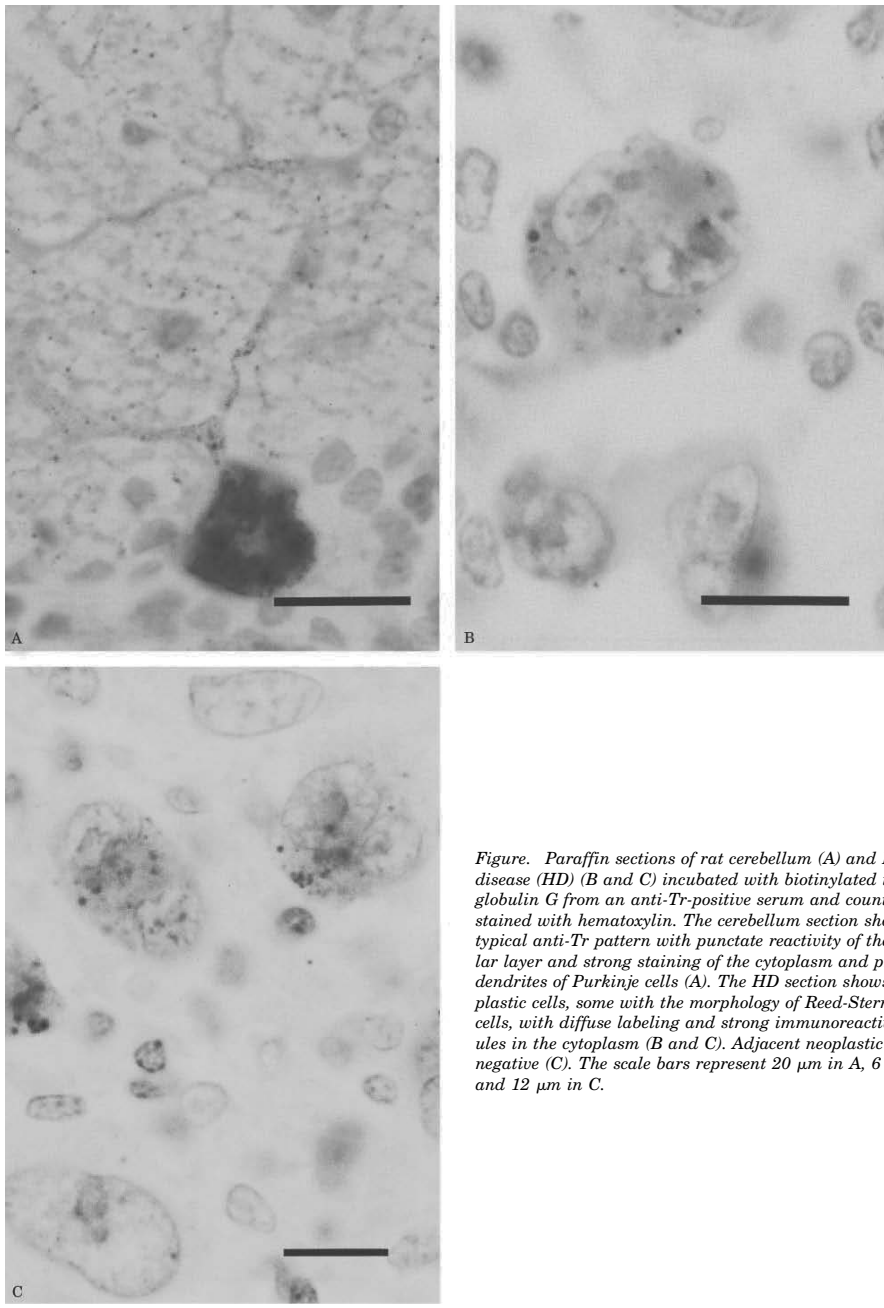

Figure. Paraffin sections of rat cerebellum (A) and Hodgkin disease (HD) (B and C) incubated with biotinylated immunoglobulin G from an anti-Tr-positive serum and counterstained with hematoxylin. The cerebellum section shows the typical anti-Tr pattern with punctate reactivity of the molecular layer and strong staining of the cytoplasm and proximal dendrites of Purkinje cells (A). The HD section shows neoplastic cells, some with the morphology of Reed-Sternberg cells, with diffuse labeling and strong immunoreactive granules in the cytoplasm (B and C). Adjacent neoplastic cells are negative (C). The scale bars represent 20 μm in A, 6 μm in B, and 12 μm in C.

gens by the tumor cells and their presentation to the immune system.[29] However, we found Tr immunoreactivity in only 1 of the 15 HD samples analyzed. Although we cannot rule out technical limitations to explain the failure to show Tr expression by the malignant cells, our data suggest that the origin of the immune response in paraneoplastic neurologic disorders in HD could be different from that associated with solid tumors. HD is characterized by the presence of a minority of malignant cells of B cell lineage, some with phenotype of Reed-Sternberg cells, and a majority of reactive mononuclear cells. The immunophenotype and cytokine pattern of the tumor-infiltrating lymphocytes are consistent with Th2-type T cells and do not favor that these T cells may recognize tumor-specific antigens.[30] At the same time, HD usually causes a polyclonal B cell activation[31] that in an appropriate setting (viral infection or a particular genetic background)[32] could induce an autoimmune attack to the cerebellum in absence of Tr antigen expression by the malignant cells. This mechanism has been proposed to explain the development of autoimmune hemolytic anemia in chronic lymphocytic leukemia. The malignant B cells, which only produce monoclonal IgM antibodies, induce, by unclear mechanisms, a polyclonal B cell activation that synthesizes the IgG autoantibodies responsible for the autoimmune hemolytic anemia.[33]

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CHAPTER 5

A NEW PARANEOPLASTIC ENCEPHALOMYELITIS AUTOANTIBODY REACTIVE WITH THE AXON INITIAL SEGMENT

Setareh Shams'ili, Bertie de Leeuw, Esther Hulsenboom,
Dick Jaarsma, Peter Sillevis Smitt

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ABSTRACT

Serum from a patient with paraneoplastic encephalomyelitis (PEM) and small cell lung cancer (SCLC) showed high titer immunohistochemical staining of the axon initial segment (AIS) on rat and human brain sections. EM studies showed that the antigen was localized in close proximity of the microtubules in the AIS. Double labeling experiments and absence of staining at the nodes of Ranvier excluded the previously identified βIV spectrin as autoantigen. Screening a rat hippocampal cDNA library resulted in the isolation of ubiquitin-conjugating enzyme E2E1 (UBE2E1). However, blocking and elution experiments excluded UBE2E1 as the AIS autoantigen.

Paraneoplastic encephalomyelitis (PEM) was first defined by Henson and colleagues as clinical or pathological dysfunction of various parts of the nervous system in patients with cancer [9]. More recently, an international panel suggested to use the term 'paraneoplastic encephalomyelitis' to describe patients with relevant clinical dysfunction at multiple levels of the central nervous system including the dorsal root ganglia and myenteric plexus [8]. The cause of PEM is believed to be immune-mediated and several onconeural antibodies have been defined in PEM that are highly specific for particular underlying tumors. The most frequently detected onconeural antibodies in PEM are anti-Hu (ANNA-1) or anti-CV2 (CRMP5) that are both strongly associated with small cell lung cancer (SCLC) [8]. However, not all patients with PEM have well defined anti-Hu or anti-CV2 onconeural antibodies, suggesting that other, as yet unknown, antigens may trigger the extensive neuronal damage. In this study, we characterize the target antigen of a new antibody in a patient with PEM and SCLC with immunoreactivity against the axon initial segment (AIS).

A 78-year-old man presented with gait instability and change of character. Over the past 4 weeks he had become withdrawn and slower. Since 2 weeks he had complex partial seizures. His mini mental state examination was 24 (out of maximum 30). Neurological examination further revealed severe gate ataxia and mild appendicular ataxia. Laboratory examination showed SIADH with Na 120 mmol/l. Onconeural antibodies (Hu, Yo, CV2, Ri, amphiphysin, Tr, and Ma2) were negative. The CSF contained 1 lymphocyte/mm^3 with normal protein concentration and negative cytology. CT-scan of the brain was normal. CT of the thorax showed a large tumor in the left upper lobe with extensive mediastinal metastases and a metastasis in the right lower lobe. Bronchoscopic biopsy revealed a SCLC. The patient was treated with one cycle of etoposide. No neurological improvement occurred and he died 3 months after presentation of the symptoms.

Immuhistochemistry was performed on paraformaldehyde-fixed frozen rat brain tissue and snap frozen, acetone fixed systemic rat tissues and human cerebellum as previously described [5,16]. For confocal microscopy studies, rat 12 μm brain sections were incubated with patient's serum (1:500) and anti βIV spectrin [2] (kindly provided by Professor M. Solimena (Dresden, Germany, 1:50) for 48 h at 4 °C and detection performed with anti-rabbit goat IgG conjugated to Cy3 (1:100) followed by anti-human FITC conjugated rabbit IgG (1:40) (both DakoCytomation, Copenhagen, Denmark). Images were obtained using a BioRad MRC station (BioRad, Hercules, CA), a Zeiss Axiovert microscope (Carl Zeiss, Sliedrecht, Netherlands) and LSM5 image processor (Carl Zeiss).

For electron microscopy (EM), anesthetized adult mice were perfused with 4% paraformaldehyde and 0.2%glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.2). Tissue was prepared for postembedding immuno-EM as described [10]. Ultrathin sections were incubated with the patient's serum (1:1000) in TBS 0.5% BSA 0.1% Triton X-100 and detection performed with 5-nm-

* Corresponding author at: Erasmus MC, Department of Neurology, Room H639, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands. Tel.: +31 10 7033327; fax: +31 10 7032156.
E-mail address: p.sillevissmitt@erasmusmc.nl (P.S. Smitt).
[1] These authors contributed equally.

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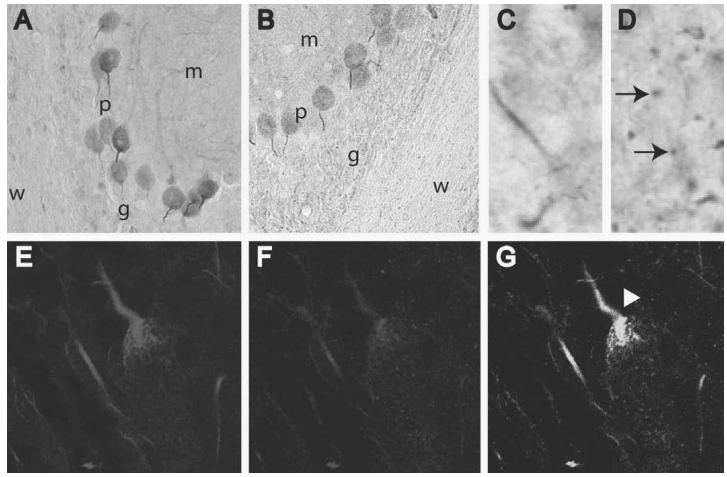

Fig. 1. Immunohistochemistry. Incubation of paraformaldehyde fixed rat cerebellar tissue with the patient's serum (A) or with a polyclonal serum against βIV spectrin (B) shows staining of the axon initial segment (AIS). In the spinal cord, the patient's serum does not stain nodes of Ranvier (C), whereas the anti-βIV spectrin antibody does (D arrows). (E–G) Confocal double staining with the serum of the patient (E; FITC, green) and anti-βIV spectrin antibody (F; Cy3, red) clearly shows that the autoantigen recognized by the patient's serum does not completely colocalize with βIV spectrin (G arrowhead). Original magnification 40× (A, B) and 100× (C–G); g, granular layer; m, molecular layer; p, Purkinje cell layer; w, white matter. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

gold-conjugated goat anti-human IgG (Aurion, Wageningen, The Netherlands, 1:30). Gold-labeled sections were contrasted with uranyl acetate and lead citrate, and analyzed in a Philips CM100 at 80 kV.

We used the patient's serum to probe a λ-ZAP Express (Stratagene, LaJolla, CA) rat hippocampus cDNA library (kindly provided by Dr. N. Galjart and Prof. C. De Zeeuw, Erasmus MC, The Netherlands). Over 10^6 plaques were screened according to the picoBlue immunoscreening kit (Stratagene) with patient's serum (1:10,000) for 12 h at 4 °C. Detection involved alkaline phosphatase conjugated anti-human IgG made in rabbit (DakoCytomation, 1:1000) and the AP-conjugate substrate kit (BioRad). Double positive clones were purified and phage clones were rescued in pBK-CMV phagemid vector using the in vivo excision phage rescue protocol (Stratagene). Insert DNA was sequenced using a LiCor automated sequencer (MWG Biotech, Ebersberg, Germany).

DNA fragments of the clones of interest were extracted, purified using QIAEX II protocol (QIAGEN, Valencia, CA) and inserted into the pET 28 high expression vector (Novagen, Madison, USA). Protein was produced in *E. coli* strain BL21(DE3)pLysS (Novagen) and purified using metal chromatography as previously described [12]. Neuronal protein extracts were prepared from fresh calf cerebellum as described [6]. Proteins were resolved by 12% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Hybond-C, Amersham, Cardiff, United Kingdom). The milk-blocked membranes were cut into strips that were incubated with diluted patient serum, polyclonal rabbit anti-UbcH6 (1:400; Boston Biochemistry, Cambridge, MA) or T7 positive control monoclonal antibody (1:10,000; Novagen). Secondary antibodies included rabbit anti-human IgG, swine anti-rabbit IgG and rabbit anti-mouse IgG, all conjugated to alkaline phosphatase (DakoCytomation). Reactivity was detected using the BioRad AP-conjugate substrate kit. For blocking experiments, the patient's serum was incubated with increasing amounts of UBE2E1 or similarly prepared Ri [3] fusion protein, as previously described [12]. In a separate experiment, the patient's serum was incubated with UBE2E1 or Ri fusion protein and the bound IgG was subsequently eluted from the blots as described [12].

The serum of the patient immune-reacted strongly (titer >1:10,000) with the axon initial segment (AIS) or axon hillock of

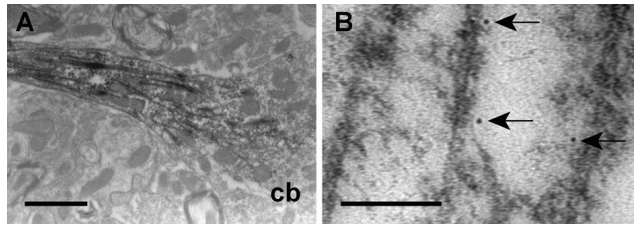

Fig. 2. Ultrastructural localization of the target antigen in motoneurons. (A) Immunogold labeled patient serum (1:1000) densely stains the AIS in close proximity of microtubules extending into the cell body (cb). (B) Close-up of microtubules in the AIS (arrows point to gold particles). Scale bar indicates 1 μm (A), 100 nm (B).

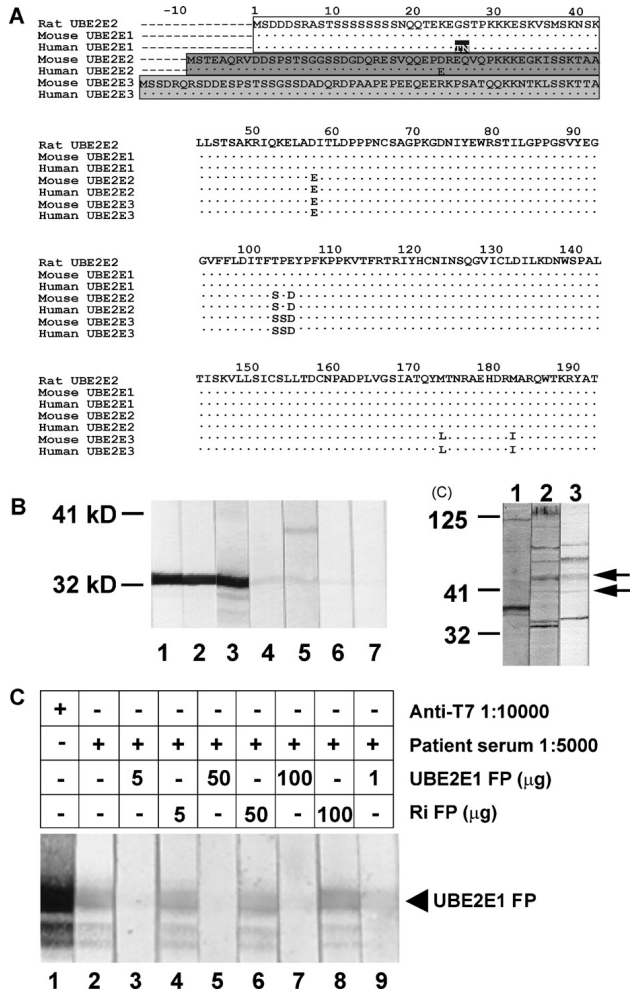

Fig. 3. Immunoreactivity with rat ubiquitin-conjugating enzyme E2E1. (A) Three independent clones from a rat hippocampus cDNA library showed highest homology to gi|50925720|gb|BC079134|, annotated as rat UBE2E2. However, this sequence is more homologous to mouse and human UBE2E1 than to UBE2E2 and is further referred to as rat UBE2E1. (B) Western blot of rat UBE2E1 fusion protein (32 kDa) stains with anti T7 (lane 1, 1:10,000), patient serum (lane 2, 1:200) and anti-UbcH6 (1:1000, lane3) but not with anti-Yo or anti-Ri patient sera (lanes 4 and 5, 1:200) or second step alone (lane 6 anti-human, lane 7 anti-rabbit, 1:1000). (C) Western blot of calf cerebellum extract shows staining of two bands at approximately 41 kDa both with patient serum (lane 2, 1:200) and anti-UbcH6 (1:200, lane 3) but not with anti-Hu patient serum (lane 1, 1:200). (D) Blocking experiments show efficient and specific blocking of patient serum with 5 or more microgram of rat UBE2E1 fusion protein. The same blocking conditions did not show visible reduction of immunofluorescent staining of the axon initial segment (not shown). FP, fusion protein.

both large and small neurons in all regions of the rat brain (Fig. 1). Frozen sections of systemic rat tissues (kidney, liver, spleen, intestine and heart) were negative (not shown).

Berghs et al. [2] previously reported a patient with paraneoplastic motor neuron disease whose serum harboured autoantibodies reactive with the AIS and with βIV spectrin. Fig. 1A and B shows the similarity of our patient's serum with βIV spectrin immunoreactivity. Although both antibodies stained the AIS, double labeling showed that the signals did not completely overlap (Fig. 1E–G). In addition, the antigen recognized by our patient's serum did not stain the nodes of Ranvier (Fig. 1C and D). Postembedding immunogold-EM (Fig. 2) indicated that the target antigen was localized at the AIS, in close proximity to microtubules. In contrast, previous ultrastructural studies localized βIV spectrin at the dense membrane undercoat of the AIS and nodes of Ranvier [11]. We, therefore, concluded that

the target antigen of the patient's serum was not βIV spectrin.

Screening of a rat hippocampus library identified three independent positive clones that all contained the entire open reading frame of rat ubiquitin-conjugating enzyme E2E1 (UBE2E1). The rat sequence that we obtained was 96% identical to BC079134.1 (designated rat UBE2E2) with the exception of a repeated segment of 56 bp inserted after the stop codon. Because BC079134.1 is more similar to UBE2E1 than to UBE2E2 proteins from other species (Fig. 3A), we concluded that BC079134.1 actually represents rat UBE2E1 rather than UBE2E2. Our cloned rat sequence differed by only two of the 193 amino acids from UbcH6, the human UBE2E1 homologue (Fig. 3A).

The serum of the patient and the commercial UbcH6 antiserum reacted strongly with recombinant purified UBE2E1 fusion protein (Fig. 3B). The serum of the patient recognized two bands of approximately 41 kDa in immunoblots of calf neuronal extracts. The antiserum against UbcH6 stained the same bands (Fig. 3C). To investigate the prevalence of UBE2E1 immunoreactivity in paraneoplastic neurological disorders (PND), we examined sera from 84 patients with neurological symptoms and suspected PND, 14 of whom had lung cancer. None of these patients' sera reacted with the UBE2E1 fusion protein (results not shown).

Pre-incubation of the patient's serum with increasing amounts of UBE2E1 fusion protein completely abolished the serum's reactivity on Western blot (Fig. 3D). However, the incubation with UBE2E1 did not affect the immunofluorescent staining of the AIS (not shown). In addition, patient IgG eluted from UBE2E1 blots did not reproduce the staining of the serum (not shown). These data indicate that the serum may contain two or more different high-titered immunoreactivities, with both the AIS and with UBE2E1.

The patient presented with symptoms of limbic encephalitis (behavioral changes, cognitive dysfunction and complex partial seizures) and cerebellar degeneration. Detection of an underlying SCLC confirmed the diagnosis of PEM [8]. Immunohistochemical studies showed that the serum of the patient contained high titer autoantibodies immunoreactive with the axon initial segment (AIS). We also examined serum samples from more than 6000 patients for the presence of paraneoplastic antineuronal antibodies. These samples included serum samples from more than 100 patients with lung cancer. Only the serum from the index patient showed immunoreactivity with the AIS. Autoantibodies reactive with the AIS and with βIV spectrin have been described once before in a breast cancer patient with a paraneoplastic motor neuron syndrome [1,2]. Based on immuno-EM and confocal double labeling studies, we could exclude βIV spectrin immunoreactivity in our patient's serum. The localization of the antigen to the AIS microtubules and the exclusion from the nodes of Ranvier also excludes Na^+ and K^+ channels and associated proteins that are highly enriched at the AIS and the nodes [14]. Instead the antigen might have a role in the specialized microtubule organization in the AIS [15].

Screening of a rat hippocampal cDNA library identified three clones with highest homology to the rat accession BC079134.1 [17], the closest human homolog of which is ubiquitin-conjugating enzyme H6 (UbcH6 or UBE2E1). Ubiquitin-mediated protein degradation is a highly conserved eukaryotic process that is comprised of well-defined steps. These involve ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s) and ubiquitin ligases (E3s) [4,7]. Although a single E1 activates the ubiquitin conjugation machinery, a large number of E2 conjugating enzymes and E3 ligases exists [4,7]. The best-characterized role of ubiquitination is to render proteins susceptible to degradation by the proteasome. Accordingly, alterations in the ubiquitination pathways contribute to the pathogenesis of many diseases including neurodegenerative disorders and cancer [4,7].

Extensive protein expression studies of UbcH6/UBE2E1 have, to our knowledge, not been reported and the commercially available rabbit anti-UbcH6 antibody does not immunoreact with tissue sections (data not shown). However, mRNA expression studies show ubiquitous expression not only in the nervous system, but also in systemic tissues and many different tumors [13]. In addition, blocking the serum of the patient with UBE2E1 fusion protein did not abrogate the immunoreactivity with the AIS antigen and IgG eluted from the UBE2E1 fusion protein did not reproduce the serum's immunoreactivity. Therefore, the patient's serum most likely contains high-titered immunoreactivity (>1:10,000) against both UBE2E1 and another autoantigen located at the AIS.

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CHAPTER 6

AN UNCONTROLLED TRIAL OF RITUXIMAB FOR ANTIBODY ASSOCIATED PARANEOPLASTIC NEUROLOGICAL SYNDROMES

Setareh Shams'ili, Janet de Beukelaar, Jan Willem Gratama, Herbert Hooijkaas, Martin van den Bent, Mars van 't Veer, Peter Sillevis Smitt

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S. Shams'ili · J. de Beukelaar · M. van den Bent · P. Sillevis Smitt, MD, PhD (✉)
Dept. of Neurology
Erasmus MC
Dr Molewaterplein 40
3015 GD Rotterdam, The Netherlands
Tel.: +31-10/463-3327
Fax: +31-10/463-3208
E-Mail: p.sillevissmitt@erasmusmc.nl

S. Shams'ili
Dept. of Neurosurgery
Erasmus MC
Rotterdam, The Netherlands

Erasmus MC
Rotterdam, The Netherlands

M. van 't Veer
Dept. of Hematology
Erasmus MC
Rotterdam, The Netherlands

Abstract Anti-CD20 monoclonal antibody (rituximab) is effectively used in the treatment of B-cell lymphomas. Recent reports in the literature suggest that antibody associated autoimmune disorders may respond to rituximab. We therefore treated nine patients with anti-Hu or anti-Yo associated paraneoplastic neurological syndromes (PNS) with a maximum of four monthly IV infusions of rituximab (375 mg/m^2). In this uncontrolled, unblinded trial of rituximab, three patients improved ≥ 1 point on the Rankin Scale (RS). One patient with limbic encephalitis improved dramatically (RS from 5 to 1). Further studies of rituximab in autoantibody associated PNS are warranted.

Key words paraneoplastic neurological syndromes · anti-Hu · anti-Yo · rituximab

Abstract Anti-CD20 monoclonal antibody (rituximab) is effectively used in the treatment of B-cell lymphomas. Recent reports in the literature suggest that antibody associated autoimmune disorders may respond to rituximab. We therefore treated nine patients with anti-Hu or anti-Yo associated paraneoplastic neurological syndromes (PNS) with a maximum of four monthly IV infusions of rituximab (375 mg/m^2). In this uncontrolled, unblinded trial of rituximab, three patients improved ≥ 1 point on the Rankin Scale (RS). One patient with limbic encephalitis improved dramatically (RS from 5 to 1). Further studies of rituximab in autoantibody associated PNS are warranted.

Introduction

Paraneoplastic neurological syndromes (PNS) are autoimmune disorders that are in approximately 50 % associated with antineuronal autoantibodies reactive with onconeural antigens [11]. Well characterized onconeural antibodies include anti-Hu (ANNA-1) [8, 19] and anti-Yo (PCA-1) [14, 17, 18]. Despite aggressive antitumor and immunosuppressive treatment, the neurological outcome in antibody associated PNS is grim and only 5–7 % of patients with Hu antibodies improve ≥ 1 point on the Rankin Scale [8, 19]. The prognosis of Yo patients is even worse: in two series of 55 and 34 patients, none of the patients improved neurologically [14, 17]. Although the precise pathogenesis of anti-Hu and anti-Yo associated PNS is unknown, it has been postulated that the antibodies or antigen-specific cytotoxic T cells [1, 2], or both, play a role [3]. A pathogenic role of the anti-Hu and anti-Yo antibodies has been suggested by high CSF titers and intrathecal synthesis of these antibodies [6] but could never be proven in animal models [7, 21].

Rituximab (Roche Ltd, Basel, Switzerland) is a chimeric anti-CD20 monoclonal antibody that is approved for the treatment of CD20+ B-cell non-Hodgkin's lymphoma. CD20 is a surface membrane antigen expressed mainly by B-cell precursors and mature B-cells and appears to play an important functional role in B-cell activation, proliferation and differentiation. In a recent randomized controlled trial in autoimmune rheumatoid arthritis, rituximab provided significant improvement in disease symptoms with concomitant lowering of rheumatoid factor [4]. Some efficacy of rit-

uximab has been reported in open-label studies in a variety of other antibody associated autoimmune disorders including polyneuropathy associated with IgM antibodies [13, 16].

We hypothesized that rituximab induced elimination of circulating B lymphocytes in patients with newly diagnosed anti-Hu and anti-Yo associated PNS would result in the prevention of the development of antibody secreting cells, in a reduction of autoantibody titers and in clinical improvement or stabilization.

Materials and methods

Patients

From February 2001, we identified nine patients with newly diagnosed antibody-associated PNS (Table 1). Eight patients had anti-Hu and one patient had anti-Yo antibodies, all at titers ≥ 3200. Five Hu patients suffered widespread paraneoplastic encephalomyelitis/sensory neuronopathy (PEM/SN) while the syndrome was more restricted in three (limbic encephalitis, cerebellar ataxia and sensory neuronopathy) (Table 1). The Yo patient suffered from typical paraneoplastic cerebellar degeneration (PCD). Six patients had a histologically confirmed neoplasm and in the other three patients a lung tumor was radiologically suspected (bronchoscopic biopsy negative). In seven patients the tumor was diagnosed only after the onset of the neurological symptoms.

Treatment

After obtaining informed consent, patients were treated with a maximum of four monthly IV infusions of rituximab (375 mg/m^2). The first dose was given as an inpatient; subsequent doses were administered in day care. Patients were pretreated with acetaminophen 1,000 mg PO and clemastine 2 mg IV to reduce the frequency of side effects. The only side effect that occurred during the infusions was transient lowering of blood pressure, which required a slower infusion rate for one of the nine patients. There were no infections despite elimination of all detectable circulating B cells in eight patients. Patients 2, 6 and 8 received concomitant standard chemotherapy for SCLC without infectious complications.

Outcome

The neurological disability was assessed using a modified Rankin scale (RS) [9]. On the modified RS a score of 0 represents an asymptomatic patient; 1, symptoms that do not interfere with lifestyle; 2, symptoms that 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 the patient does not need constant attention; 5, severe disability with total dependence requiring constant attention; 6, death from neurological cause. A patient was considered functionally improved if there was a decrease of at least 1 point in RS measured after completing 16 weeks of treatment compared with the RS just prior to the first rituximab dose. The treatment was stopped when a patient deteriorated neurologically or when the RS increased by 1 point or more. The functional outcome was considered 'successful' when a patient with RS ≤ 3 improved or stabilized (i. e. remained ambulatory) and when a patient with RS ≥ 4 (bedridden patient) improved to ≤ 3 (ambulatory), as defined by Keime-Guibert et al. [10].

Laboratory evaluations

IgG and IgM titers of the paraneoplastic antibodies were determined on rat cerebellar sections by peroxidase immunohistochemistry and by Western blotting using purified recombinant HuD and CDR62 antigens, as described [5, 11]. Serum and CSF were sampled prior to each cycle of rituximab. Multiple samples from the same patients were titrated in a single experiment by serial endpoint dilutions on rat cerebellar sections. A difference of 2 or more dilution steps was considered significant. Absolute numbers of circulating B lymphocytes were determined prior to each cycle of rituximab by a single-platform flow cytometric assay as detailed elsewhere [15]. In that assay, B lymphocytes were identified by the CD19 marker, whose expression was not affected by rituximab therapy.

To find further evidence for a pathogenic role of antibodies or cytotoxic T lymphocytes, we measured cytokines that are involved in the Th1 or Th2 immune response. The cytokines IFN-γ, TNFα, IL10, IL-2, IL-4 and IL-5 were measured in serum and CSF with the cytokine bead array (Beckton Dickinson, San Diego, CA). Normal serum values for all cytokines tested were < 15 pg/mL.

Results

In all patients the neurological symptoms progressed in the two weeks prior to study entry. Eight of the patients had not received any immunosuppressive therapy while patient 8 had been treated unsuccessfully with prednisone (60 mg per day) for two weeks prior to start of rituximab. Three patients received standard chemotherapy for small cell lung cancer (SCLC) at the same time that they were treated with rituximab. All three achieved a complete tumor remission (Table 1) and no adverse effects of the combination treatment were observed. Patient 5 was still in CR when she developed PNS and was treated with rituximab.

Following rituximab, three patients (# 2, 4 and 8) improved functionally as indicated by a decrease in RS of one point or more (Table 2). Patient 2 presented with seizures. After control of the seizures with phenytoin, he continued to deteriorate and finally suffered complete loss of short-term memory, was severely disoriented and required constant attention (RS = 5). After diagnosis of paraneoplastic limbic encephalitis, a SCLC was found. One week after the first cycle of chemotherapy, he received rituximab. Four weeks later, his neurological condition had greatly improved (RS = 1). He was discharged home and returned to a completely independent life. He completed 4 monthly cycles of rituximab and phenytoin was stopped without recurrence of seizures. Patient 4 developed PCD shortly after diagnosis of a lymph node metastasis in the groin, probably from an undetected ovarian cancer. She was bedridden at the start of treatment (RS = 4). Following rituximab, she regained the ability to walk a block around the house and she finished 4 cycles (RS = 3). Patient 8 developed sensory and motor neuronopathy and cerebellar ataxia. PEM/SN was diagnosed and a SCLC was detected. She was treated with chemotherapy and rituximab and regained the ability to walk with support (RS from 4 to 3).

Table 1 Patient characteristics

Patient No	Age/Sex	Antibody	Syndrome	Sympt.-Diagnosis (months)	Tumor	Tumor – Sympt. (months)	Tumor – Diagnosis interval (months)	Tumor treatment	Response tumor
1	68/M	Hu	PCD	8	Prostate	−107	−115	Hormonal	PD
2	59/M	Hu	PLE	2	SCLC	2	0	Chemo and RT	CR
3	55/F	Hu	PEM	9	Lung (CT, PET)	9	0	No	–
4	48/F	Yo	PCD	1	Ovarian	0	−1	Surgery	PR
5	58/F	Hu	BE/PSN	1	SCLC	−6	−7	Chemo and RT	CR
6	56/F	Hu	PSN	4	SCLC	5	1	Chemo	CR
7	69/M	Hu	PEM/SN	3	Lung (PET)	5	2	No	
8	52/F	Hu	PEM/SN	2	SCLC	3	1	Chemo and RT	CR
9	80/M	Hu	PEM/SN	8	Lung (CT)	8	0	No	–

PCD paraneoplastic cerebellar degeneration; *PLE* paraneoplastic limbic encephalitis; *PEM* paraneoplastic encephalomyelitis; *BE* brainstem encephalitis; *PSN* paraneoplastic sensory neuronopathy; *SCLC* small cell lung cancer; *RT* radiotherapy; *PD* progressive disease; *CR* complete remission; *PR* partial remission

Because of the severity of the remaining symptoms (painful sensory neuronopathy), she declined the fourth cycle of rituximab. Three patients deteriorated, two of whom died from the neurological syndrome, and three remained stable. The functional outcome was considered 'successful' in 5 of 9 patients, as defined by Keime-Guibert et al. [10].

Following rituximab, circulating CD19+ B cell levels became undetectable (i. e., < 1 cell/µl) in 8 of 9 patients during the entire study period and were severely reduced in the other patient. Despite the successful elimination of circulating B cells, a significant decrease in serum IgG titer was detected in only 2 patients while the titer increased in one patient and remained stable in 6 patients (Table 2). In 6 patients, CSF titers after at least one cycle of rituximab were obtained. Only the CSF from patient 8 demonstrated a significant decrease in titer (from 64 to 8) with persisting high serum titer (Table 2). The CSF titer increased in patient 5 and remained stable in the other 4 patients.

Because of the lack of correlation between clinical response and IgG antibody titers, we further studied the IgM anti-Hu and anti-Yo titers. In 7/8 patients, we could detect anti-Hu IgM whereas no anti-Yo IgM reactivity was observed in patient 4. In patient 2, the serum IgM titer dropped from 12800 to 1600. In all other patients, the IgM titers remained stable at relatively low levels or decreased non-significantly. In patient 2, the serum cytokines IFNγ (261 pg/ml), IL-2 (3820 pg/ml) and IL-5 (180 pg/ml) were elevated while TNFα, IL-10 and IL-4 were normal (< 15 pg/ml). In all other patients serum cytokine levels were in the normal range as were all CSF cytokines measured.

Discussion

Three out of 9 PNS patients treated with rituximab had functional improvement as indicated by a decrease of at least one point on the Rankin scale. In 5 out of 9 patients the functional outcome was considered 'successful' according to the Keime-Guibert et al. criteria [10]. In pre-

Table 2 Neurological outcome and IgG autoantibody titers in serum and CSF following rituximab treatment

Patient No.	Antibody	No. cycles rituximab	Outcome	RS start	Change RS	Serum IgG titer		CSF IgG titer	
						start	end	start	end
1	Hu	1	Stable	3	0	3200	3200	8	16
2	Hu	4	Improved	5	−4	6400	1600	200	NA
3	Hu	2	Died	4	2	3200	3200	64	128
4	Yo	4	Improved	4	−1	6400	12800	128	64
5	Hu	2	Worse	4	1	12800	12800	32	128
6	Hu	1	Died	3	3	3200	1600	32	NA
7	Hu	3	Stable	3	0	3200	25600	2048	1024
8	Hu	3	Improved	4	−1	12800	12800	64	8
9	Hu	1	Stable	4	0	51200	3200	32	NA

RS Rankin score; *NA* not available

vious studies that use the same outcome criteria, 0–7 % of patients with anti-Hu associated PNS improved one point or more on the RS while 22–31 % had a successful outcome [10, 19]. In anti-Yo associated PNS, 0–5 % of patients improved on the RS while 10–14 % had a successful outcome [17, 18]. These results suggest that rituximab may have some effect in antibody associated PNS although the numbers are too small for a definite conclusion. However, alternative explanations for the favorable outcome in our patients are likely and include concomitant anti-tumor treatment, early start of treatment and spontaneous improvement. Several studies have demonstrated that effective treatment of the tumor is important to at least stabilize anti-Hu associated PNS [8, 9, 19]. The improvement in two of our anti-Hu patients may have been confounded by the complete tumor remission achieved during the study period. Spontaneous remission of anti-Hu associated limbic encephalitis has been described [19] and could also have occurred in patient 2, who improved dramatically following rituximab. In all three patients who improved, the PNS diagnosis was established within 2 months of the onset of symptoms and was followed shortly by start of treatment. Because most PNS ultimately result in the destruction of neurons [3], early diagnosis and treatment are crucial.

The pathogenesis of PNS is heterogenic [3]. While some PNS such as Lambert-Eaton myasthenic syndrome and a rare form of PCD [20] are clearly caused by pathogenic autoantibodies directed at cell surface epitopes, such a role could never be proven for anti-Hu and anti-Yo antibodies that are directed at intracellular antigens [22]. Rituximab administration resulted in successful elimination of circulating CD19 + B cells. However, no consistent effect on serum or CSF antibody titers was observed and we did not find any correlation between clinical response and antibody titers. These observations raise the question whether rituximab did contribute to the functional improvement observed in three patients and, if so, by which mechanism. The detection of antigen-specific cytotoxic T cells in anti-Hu and anti-Yo associated PNS [1, 2] suggests that the cellular immunity may play a pathogenic role.

Rituximab induced elimination of circulating B-cell results in significant clinical improvement in rheumatoid arthritis [4]. In this disorder B-cells may function as antigen-presenting cells and are important for T-cell activation. However, such roles for B-cells have not been demonstrated in PNS.

The improvement in 3 of 9 PNS patients following rituximab treatment warrants further studies. Because rituximab does not easily cross the blood brain barrier [12], concomitant intrathecal administration may enhance its efficacy in the treatment of PNS of the central nervous system.

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CHAPTER 7

SUMMARY AND DISCUSSION

SUMMARY AND DISCUSSION

Paraneoplastic neurologic syndromes (PNS) are rare disorders caused by remote effects of cancer. **Chapter 2** provides a detailed review of these syndromes including clinical and immunological characterizations. Newer developments since publication of chapter 2 are discussed in **Chapter 1**. PNS may involve one or more of any part of the central and peripheral nervous system, including the neuromuscular junction and muscle. Classic paraneoplastic syndromes include encephalomyelitis, limbic encephalitis (LE), subacute cerebellar ataxia, opsoclonus – myoclonus (OM), subacute sensory neuronopathy, Lambert-Eaton myasthenic syndrome (LEMS), chronic gastrointestinal pseudo-obstruction and dermatomyositis Because these syndromes are preferentially associated with certain tumors, a careful and directed search for an underlying neoplasm is recommended. Antineuronal antibodies have a role in the early diagnosis of an often subacute and severe neurological syndrome as paraneoplastic and may direct the search towards an as yet undiagnosed underlying neoplasm. Anti-Hu (ANNA-1), anti-Yo (PCA-1), anti-Ri (ANNA-2), anti-amphiphysin, anti-CV2 (anti-CRMP5), anti-recoverin and anti-Ma/Ta are well characterized onconeural antibodies. Recently it is proposed to categorize the antineuronal antibodies in two main groups based on location of the corresponding antigen. Antibodies against intracellular antigens, including the well characterized onconeural antigens, are non-pathogenic and serve as biomarkers of a probably T cell mediated neuronal destruction. On the other hand, antibodies against cell surface antigens often cause functional inhibition of the target antigen and may be pathogenic. Clinical syndromes caused by these antibodies are more responsive to immunotherapy. Previously LE was believed to be almost always paraneoplastic. More recently, the detection of antibodies against cell surface neuronal antigens (anti-NMDAR, anti-AMPA, anti-VGKC and anti-GABAB) has allowed the diagnosis of an autoimmune form of LE that is often not associated with an underlying neoplasm.
The cornerstone of treatment of PNS remains the eradication of the associated tumor by anti-cancer modalities. In addition, various immunotherapeutic treatments are also tried with limited success.

One of the most common classic PNS is paraneoplastic cerebellar degeneration (PCD). In **chapter 3**, we present a retrospective study of over 5000 blood samples obtained from patients with a neurological syndrome suspected of having a paraneoplastic etiology. Our study confirmed that definite PNS are rare since only 137 patients with PNS and a high titer (>400) onconeural antibody were found out of ~5000 samples (~3%). The frequency of antineuronal antibodies detected in this study and their related clinical syndromes are summarized in Table 2 of this chapter. Fifty (36%) had PCD and 84% of these patients proved to have an associated neoplasm which in the majority (62%) was diagnosed only after the cerebellar dysfunction was diagnosed. This finding confirms and emphasizes the role of antineuronal antibodies in the diagnosis of a paraneoplastic syndrome. The most common tumors associated with PCD were lung and gynecological cancers. The main factors affecting overall survival were administration of antitumor treatment

and younger age; however, these findings were not statistically significant. The survival analysis could be done for 48 PCD patients and is presented in Figure 1 of chapter 3. In our study, the cause of death in anti-Yo patients, was mostly (67%) the neurological dysfunction. In contrast, Rojas et al. (2000) reported the cause of death to be a neurological syndrome in only 29% of anti-Yo patients with most patients dying of tumor progression. This difference was not explained by the associated malignancies. However, documentation of cause of death might be different among treating physicians, as some of the deaths due to a neurological dysfunction associated with a non responsive or progressive malignancy, might have been documented as progressive disease related to the underlying malignancy. In addition, these are retrospective studies largely preventing harmonization of documentation among investigators. Future prospective studies might be needed to resolve these and many other issues in PNS.

Hodgkin's disease (HD) is the third most common malignancy associated with PCD. Initial studies have suggested that anti-Tr antibodies identify patients with PCD and HD. As described in chapters 1 and 2 of this thesis, anti-Tr antibodies are partially characterized because the target antigen has not yet been identified. In collaboration with the group of Graus, we performed a retrospective study with the objectives to confirm the association of this autoantibody with HD and PCD and to improve its characterization. **Chapter 4** describes this clinical and immunological analysis of 28 patients positive for anti-Tr antibody. Twenty-five were found to have a malignancy, all diagnosed as HD. There was only one patient without cerebellar ataxia and this patient showed clinical features of LE. Hereby we confirmed the strong association of anti-Tr antibodies with both PCD and HD. In addition, we established that the anti-Tr antibodies from various patients recognized the same epitopes, indicating limited epitope usage by the anti-Tr antibody. Therefore, the immunocompetition assay we used in this study can be used as confirmatory test of anti-Tr reactivity. Autopsy of one of the anti-Tr positive patients showed total destruction of Purkinje cells. Immunohistochemistry disclosed only CD3 and sporadic CD68 positivity, suggesting that T cell mediated autoimmunity is potentially involved in the process of CNS inflammation and neuronal destruction. We also showed that the major subclasses of anti-Tr are IgG1 and IgG3, a finding which is line with other autoantibodies. It is proposed that this predominance is related to a Th1-type response of CD4+ T helper cells reactive with the antigen. Several findings in our study of anti-Tr are different from those previously reported for other antineuronal antibodies. First, only one of 15 available associated HD tumor samples showed anti-Tr positivity. In the one positive HD sample, the Tr-immunoreactivity was limited to only some Reed-Sternberg cells with mainly cytosolic staining. In contrast, all well characterized onconeural antibodies react with antigens expressed by both the tumor and affected parts of the nervous system. Second, the anti-Tr antibodies disappeared spontaneously in the three patients who did not have a tumor and in all the patients who achieved a tumor response. Disappearance over time has not previously been reported with other paraneoplastic antibodies. This difference among patients with a tumor might be explained by a higher efficacy of antineoplastic treatment used to control

HD resulting in elimination of the target antigen, in comparison to other solid tumors. In addition, successful treatment of HD (a B-cell lymphoma) results in decrease or disappearance of B-cell clones. The spontaneous disappearance of anti-Tr antibodies in all three patients without HD might suggest that in some cases, the origin of anti-Tr antibody is not the malignancy, and antibody generation might have been triggered by other undetected causes, such as a viral infection. Third, anti-Tr antibodies were absent in the serum but positive in the CSF of two patients. This finding is not reported with other onconeural antibodies and has important clinical implications.

Identification of new onconeural antibodies and their corresponding antigens improves early diagnosis of PNS and might have a positive impact on neurological functional outcome and cancer survival by proceeding to an earlier and appropriate treatment. In **chapter 5**, we describe our finding of a patient with paraneoplastic encephalomyelitis (PEM) associated with a small cell lung cancer (SCLC) whose serum showed a distinct and unreported immunostaining pattern. Subsequently we attempted to characterize the related target antigen. The results of testing the serum of this patient for known antineuronal antibodies were negative but immunohistochemistry (IHC) showed staining of the axon initial segments (AIS) in rat and human CNS tissues. In order to define the frequency of such a pattern, we screened serum samples of more than 6000 patients suspected to have a PNS, including also 100 patients with lung cancer. Only the serum from the described patient showed immunoreactivity with the AIS. We excluded other previously reported antigens with a similar IHC pattern, by using electron microscopy and double labeling experiments. These antigens included Beta-IV spectrin (reported in one patient with breast cancer and motor neuron syndrome) and Na+ and K+ channels and associated proteins. By screening a rat hippocampal cDNA library, we isolated an ubiquitin-conjugating enzyme, UBE2E1, homologous to human UbcH6. However, blocking and elution experiments did not confirm UBE2E1 as the AIS autoantigen. But immunoblotting experiments showed that the patient's serum and the commercial UbcH6 antibody both reacted strongly with recombinant purified UBE2E1 fusion protein, recognizing the same bands of approximately 41 kDa. We concluded that our patient's serum contained high titer antibodies against both UBE2E1 and another autoantigen located at the AIS. We could not conclude whether the antibodies against UBE2E1 were related to the patient's lung cancer or could be a shared antigen between the lung tumor and CNS.

Ubiquitination pathways are involved in neural development, plasticity, and degeneration[1] as well as in cancer. The ubiquitin pathway plays an important role in synaptic function and may contribute to disease-induced changes. Disturbances of activity of protein components of ubiquitination system, including specific E2 and E3 molecules, might result in significant disruption of protein trafficking and neuronal connectivity[1-3]. It is shown that UbcH6 modulates the transcriptional repression activity of ataxin-1. Ataxin-1 accumulation in cerebellar Purkinje cells results in damage of these cells in spinocerebellar ataxia type 1 (SCA1), a rare neurodegenerative disorder[4-5]. In cancer, UbCH6 is involved in regulation

of a tumor suppressor candidate, TSSC5[5-6] At least some members of ubiquitin conjugating enzymes family have the ability to promote cell growth and malignant transformation[7] or are overexpressed in some cancers such as breast tumors[8]. Future studies are warranted to improve the understanding of the functional role of ubiquitination pathways and proteins, including UBE2E1, both in cancer and neurological disorders.

The AIS contains three distinct structures, including fascicles of microtubules, membrane undercoating and clusters of ribosomes[9]. Fascicles of microtubules exist only in the axon hillock and initial segment and nowhere else in the neuron. A specific function of AIS microtubular structure is not clear although it is suggested that AIS cytoskeleton regulates neuronal polarity[10-11]. There are not many proteins identified in AIS. The antigen that our patient's serum reacted to is located in AIS microtubules. Characterization of the target antigen of this antibody which produces staining of the AIS, but not nodes of Ranvier, might add to diagnostic PNS antibody profiling and may be of general interest to neuroscience. AIS is an important neuronal structure, being both anatomically and physiologically a bridge between soma and axonal domains, responsible for initiating and modulating action potentials[12-13] . In addition, genetic loss of the AIS cytoskeleton disturbs neuronal function and organization. Recently it was shown that neuronal injury causes irreversible proteolysis of the AIS cytoskeleton independently of cell death or axon degeneration, leading to disruption of the molecular organization of the AIS and disrupted neuronal polarity[14]. Identification of the target antigens of autoantibodies reactive with the AIS may facilitate future studies directed at improving our understanding of the molecular structure and function of the AIS.

Treatment of PNS is an unmet medical need. As discussed in chapter 1, there is increasing support for the involvement of both humoral and cellular immune mechanisms in the pathogenesis of PNS. We hypothesized that reduction of autoantibody titers at an early stage may stabilize the disease. Subsequently we hypothesized that elimination of circulating B lymphocytes, might reduce secretion of autoantibodies into the circulation and diminish ongoing antigen presentation. Rituximab is a chimeric anti-CD20 monoclonal antibody that causes rapid and long-lasting elimination of circulating B cells. Rituximab is approved to be used alone or with other medications in certain types of CD20 positive B cell non-Hodgkin's lymphomas and in chronic lymphocytic leukemia. It is also approved for treatment of refractory rheumatoid arthritis. **Chapter 6** describes our clinical trial with Rituximab in nine patients with PNS associated with high titer antineuronal antibodies (eight with anti-Hu and one with anti-Yo). The anti-Yo positive patient with an ovarian cancer, and two of the anti-Hu positive patients, both with a SCLC, showed improvement of neurological function. Even though a successful elimination or significant reduction of circulating B cells was observed in all patients, no consistent decrease of serum or CSF antibody titers was observed. Considering the limited response to immunotherapy in PNS, the improvement of three out of nine patients in this study suggests some positive effects of rituximab in this patient population. However, the size of study was very small and there were

confounding factors such as concomitant cytotoxic therapy that makes an accurate conclusion difficult. In addition, to increase the exposure of CNS to rituximab, intrathecal administration of this drug may be tried.

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CHAPTER 8

SAMENVATTING EN DISCUSSIE

(Dutch)

SAMENVATTING EN DISCUSSIE

Paraneoplastische neurologische syndromen (PNS) zijn zeldzame aandoeningen die indirect door een onderliggende tumor veroorzaakt worden. **Hoofdstuk 2** geeft een gedetailleerd overzicht klinische en immunologische aspecten van deze syndromen. Nieuwere ontwikkelingen sinds de publicatie van Hoofdstuk 2 worden besproken in **Hoofdstuk** 1. Bij PNS kan één of meer van enig deel van het centrale en perifere zenuwstelsel, met inbegrip van de neuronmusculaire overgang en spieren zijn aangedaan. Klassieke paraneoplastische syndromen omvatten encefalomye1itis, limbische encefalitis (LE), subacute cerebellaire ataxie, opsoclonus - myoclonus (OM), subacute sensibele neuronopathie, Lambert-Eaton myastheen syndroom (LEMS), chronische gastro-intestinale pseudo-obstructie en dermatomyositis. Omdat deze syndromen bij voorkeur met bepaalde tumoren geassocieerd zijn, is een zorgvuldig en gericht zoeken naar een onderliggende maligniteit aanbevolen. Antineuronale antilichamen diagnosticeren in een vroeg stadium een vaak subacuut en ernstig neurologisch syndroom als paraneoplastisch en kunnen de zoektocht naar een meestal nog niet bekende onderliggende maligniteit richting geven. Anti-Hu (ANNA-1), anti -Yo (PCA-1), anti-Ri (ANNA-2), anti-amfifysine, anti-CV2 (anti-CRMP5), anti-recoverine en anti-Ma/Ta zijn goed gekarakteriseerde onconeurale antilichamen. Onlangs is voorgesteld om de antineuronale antilichamen te categoriseren in twee hoofdgroepen gebaseerd op de locatie van het desbetreffende antigen. Antistoffen tegen intracellulaire antigenen, waaronder de goed gekarakteriseerde onconeurale antigenen, zijn niet pathogeen en dienen als biomarkers voor een waarschijnlijk T cel gemedieerd verlies van neuronen. Antilichamen gericht tegen celoppervlakte antigenen interveniëren vaak met de functie van deze antigenen en kunnen zo pathogeen zijn. Klinische syndromen veroorzaakt door deze antilichamen reageren in het algmeen beter op immunotherapie. Voorheen werd LE geacht bijna altijd paraneoplastisch te zijn. Door de recente ontdekking van antilichamen tegen neuronale celoppervlakte antigenen (anti-NMDAR, anti-AMPA, anti-VGKC en anti-$GABA_B$) is gebleken dat er ook een auto-immuun vorm van LE bestaat die vaak niet geassocieerd is met een onderliggende maligniteit.
De hoeksteen van de behandeling van PNS is de behandeling van de onderliggende tumor. Daarnaast zijn verschillende immunotherapeutische behandelingen geprobeerd met beperkt succes.

Eén van de meest voorkomende klassieke PNS is paraneoplastische cerebellaire degeneratie (PCD). In **hoofdstuk 3** presenteren we een retrospectieve studie van meer dan 5000 bloedmonsters van patiënten met een neurologisch ziektebeeld met mogelijk een paraneoplastische etiologie. Onze studie bevestigt dat 'zekere' PNS zeldzaam zijn, aangezien slechts 137 patiënten met een PNS en hoge titer (> 400) onconeurale antilichamen werden gevonden onder de ~5000 monsters (~3%). De frequentie van antineuronale antistoffen aangetoond in deze studie en de bijbehorende klinische syndromen zijn samengevat in tabel 2 van dit hoofdstuk. Vijftig (36%) had PCD en 84% van deze patiënten bleek een geassocieerde maligniteit te hebben waarvan in de meerderheid (62%) de diagnose pas na de

cerebellaire disfunctie werd vastgesteld. Deze bevinding bevestigt en benadrukt de rol van antineuronale antistoffen in de diagnose van een paraneoplastisch syndroom. De meest voorkomende tumoren bij PCD zijn long- en gynaecologische kankers. De belangrijkste factoren die van invloed waren op totale overleving van PCD patiënten zijn de toediening van antitumorale behandeling en jongere leeftijd, maar deze bevindingen waren niet statistisch significant. De survival analyse kon worden gedaan voor 48 PCD patiënten en wordt gepresenteerd in figuur I van hoofdstuk 3. In onze studie was de neurologische disfunctie de doodsoorzaak in de meerderheid van de anti-Yo patiënten (67%). Rojas et al. (2000) meldden daarentegen dat de meeste anti-Yo patiënten overleden ten gevolge van de tumor en slechts 29% door het neurologische syndroom. Dit verschil werd niet verklaard door de geassocieerde maligniteiten. Echter, inschatting van de doodsoorzaak kan verschillen tussen behandelende artsen. Sommige sterfgevallen als gevolg van een neurologische disfunctie, geassocieerd met een niet-responsieve of progressieve maligniteit, zouden kunnen zijn gedocumenteerd als veroorzaakt door progressie van de onderliggende maligniteit. Bovendien zijn dit retrospectieve studies waarbij harmonisatie van de documentatie door verschillende onderzoekers niet mogelijk is. Toekomstige prospectieve studies zijn nodig om deze en vele andere onderwerpen in PNS op te lossen.

De ziekte van Hodgkin (HD) is de derde meest voorkomende maligniteit geassocieerd met PCD. Eerdere studies hebben gesuggereerd dat anti-Tr antilichamen voorkomen bij patiënten met PCD en HD. Zoals beschreven in de hoofdstukken 1en 2 van dit proefschrift, zijn anti-Tr antilichamen gedeeltelijk gekarakteriseerd omdat de target antigenen nog niet geïdentificeerd zijn. In samenwerking met de groep van Graus, hebben we in een retrospectieve studie de associatie van anti-Tr met HD en PCD bevestigd en de karakterisering ervan verbeterd. **Hoofdstuk 4** beschrijft deze klinische en immunologische analyse van 28 patiënten positief voor anti-Tr. Vijfentwintig patiënten bleken een maligniteit te hebben, alle HD. Er was slechts één patiënt zonder cerebellaire ataxie en deze patiënt bleek klinische kenmerken te hebben van LE. Hiermee hebben we de sterke associatie van anti-Tr met zowel PCD als HD bevestigd. Daarnaast hebben wij vastgesteld dat de anti-Tr antistoffen van verschillende patiënten dezelfde epitopen herkennen, wijzend op beperkt epitoop gebruik. Daarom is de immuun competitie test gebruikt in deze studie in te zetten om anti-Tr reactiviteit te bevestigen. Obductie van één van de anti-Tr positieve patiënten toonde compleet verlies van Purkinje cellen. Immunohistochemie liet uitsluitend CD3 en sporadische CD68 positiviteit zien. Dit suggereert dat T cel gemedieerde auto-immuniteit mogelijk betrokken is bij het proces van ontsteking en neuronale destructie. We toonden ook aan dat IgG1 en IgG3 de belangrijkste subklassen van anti-Tr zijn, net als bij andere auto-antilichamen, wijzend op een Th1 type respons van CD4+ T helper cellen reactief met het antigen. Verschillende bevindingen in onze anti-Tr studie verschillen van die welke eerder gemeld zijn voor andere onconeurale antilichamen. In de eerste plaats toonde slechts één van de 15 beschikbare HD tumoren anti-Tr positiviteit. In het ene positieve HD monster, was de Tr-immunoreactiviteit beperkt tot slechts enkele Reed-Sternberg cellen met voornamelijk cytoplasmatische kleuring. De goed

gekarakteriseerde onconeurale antistoffen reageren daarentegen met antigenen die tot expressie worden gebracht door zowel de geassocieerde tumor als de aangetaste delen van het zenuwstelsel. In de tweede plaats, de anti-Tr antilichamen verdwenen spontaan bij de drie patiënten zonder tumor en in alle patiënten die een tumor respons hadden. Verdwijning in de loop van de tijd is nog niet eerder gemeld met andere paraneoplastische antilichamen. Dit verschil tussen patiënten met verschillende antistoffen en tumoren kan wellicht worden verklaard door de hogere werkzaamheid van antineoplastische behandelingen in HD resulterend in eliminatie van de target antigenen, in vergelijking met andere solide tumoren. Bovendien, een succesvolle behandeling van HD (een B-cel lymfoom) resulteert in een afname of verdwijnen van de B-cel klonen. De spontane verdwijning van anti-Tr antilichamen in alle drie de patiënten zonder HD zou kunnen suggereren dat in sommige gevallen, de oorsprong van de anti-Tr antilichaam niet de primaire tumor is, en antilichaam generatie zou kunnen zijn veroorzaakt door andere oorzaken, zoals een virale infectie. Ten derde, anti-Tr antilichamen waren afwezig in het serum, maar positief in de liquor van twee patiënten. Deze bevinding is niet gemeld bij andere onconeurale antilichamen en heeft belangrijke klinische implicaties.

Identificatie van nieuwe onconeurale antigenen verbetert de vroegtijdige diagnose van een PNS en zou een positief effect kunnen hebben op de neurologische functie en overleving door een eerdere behandeling. In **Hoofdstuk 5,** beschrijven we onze bevinding van een patiënt met paraneoplastische encephalomyelitis (PEM) geassocieerd met een kleincellig longcarcinoom (KCLC) van wie het serum een duidelijk en niet eerder gerapporteerd immunohistochemisch patroon gaf op ratcerebellum. Vervolgens hebben we geprobeerd om het bijbehorende target antigen te karakteriseren. De resultaten van het testen van het serum van deze patiënt voor bekende antineuronale antistoffen waren negatief, maar immunohistochemie liet aankleuring zien van de axonale initiële segmenten (AIS) in CZS weefsel van rat en mens. Om de frequentie van een dergelijk immunohistochemisch patroon te bepalen, hebben we de serummonsters onderzocht van meer dan 6000 patiënten met een verdenking PNS, onder wie ook 100 patiënten met longkanker. Alleen het serum van de beschreven patiënt toonde immunoreactiviteit met AIS. We sloten andere, eerder gerapporteerde, antigenen met een vergelijkbaar immunohistochemisch patroon uit door gebruik te maken van elektronenmicroscopie en dubbelkleuringen. De geteste antigenen waren BetaIV spectrine (gerapporteerd bij een patiënt met borstkanker en motor neuron syndroom) en Na+ en K+ kanalen en geassocieerde eiwitten. Middels screening van een rat hippocampus cDNA bibliotheek, isoleerden we een ubiquitine conjugerend enzym, UBE2El, homoloog aan menselijk UbcH6. Echter, met blokkeer- en elutie-exprimenten konden we niet bevestigen dat UBE2El het AIS autoantigen was. Daarentegen toonden immunoblotting experimenten aan dat zowel serum van de patiënt als het commerciële UbcH6 antilichaam sterk reageerden met recombinant gezuiverd UBE2El fusie eiwit. Wij hebben geconcludeerd dat het serum van onze patiënt hoge titers bevatte van antistoffen tegen zowel UBE2El als een ander autoantigen gelegen in het AIS. We konden niet vaststellen of de antistoffen tegen UBE2E1 gerelateerd waren aan de longtumor van de patiënt of dat er sprake is van

een antigen dat zowel in het zenuwstelsel als de tumor tot expressie komt.

Ubiquitinering is betrokken bij neurale ontwikkeling, plasticiteit en degeneratie[1] evenals bij kanker. Ubiquitine speelt een belangrijke rol in de synaptische functie en kan bijdragen aan door ziekte geïnduceerde veranderingen. Verstoring van de activiteit van eiwitten die bij ubiquitinering betrokken zijn, zoals E2 en E3, zou kunnen leiden tot stoornissen in het eiwit transport en neuronale connectiviteit[1-3]. Het is aangetoond dat UbcH6 de transcriptionele repressie activiteit van ataxine-1 moduleert. Ataxine-1 accumulatie in de cerebellaire Purkinje cellen resulteert in een verlies van deze cellen in spinocerebellaire ataxie type 1 (SCA1), een zeldzame neurodegeneratieve aandoening[4-5]. In kanker is UbCH6 betrokken bij de regulatie van TSSC5, een kandidaat tumor suppressor[5-6]. Tenminste een aantal leden van de familie van ubiquitine conjugatie enzymen kunnen celgroei en maligne transformatie bevorderen[7]of komen verhoogd tot expressie in sommige vormen van kanker zoals borsttumoren[8].Toekomstige studies zijn gerechtvaardigd om het inzicht in de functionele rol van ubiquitinatie 'pathways' en eiwitten, met inbegrip van UBE2El te verbeteren, zowel bij kanker als bij neurologische aandoeningen.
Het AIS bevat drie verschillende structuren waaronder bundels van microtubuli, membraan
onderlagen en clusters van ribosomen[9]. Bundels van microtubuli komen alleen voor in de axon heuvel
en eerste segment en nergens anders in het neuron. Een specifieke functie van de AIS microtubulistructuur is niet duidelijk maar het is gesuggereerd dat het de neuronale polariteit regelt[10-11].Er zijn niet veel eiwitten geïdentificeerd in AIS. Het antigen waarmee het serum van onze patiënt heeft gereageerd, is gelegen in AIS microtubuli. Karakterisering van de target antigenen van deze antistof die wel het AIS aankleurt, maar niet de knopen van Ranvier, kan mogelijk bijdragen aan de diagnostiek van PNS en is wellicht van algmeen belang voor de neurowetenschappen. AIS is een belangrijke neuronale structuur, die zowel anatomisch als fysiologisch een brug vormt tussen soma en axonale domeinen, en verantwoordelijk is voor het initiëren en moduleren van actiepotentialen[12-13]. Bovendien leidt genetisch verlies van AIS tot verstoring van neuronale functie en organisatie. Recent is aangetoond dat neuronale schade proteolyse veroorzaakt van het cytoskelet van het AIS resulterend in verstoring van de moleculaire organisatie van de AIS en een verstoorde neuronale polariteit[14]. Identificatie van de target antigenen van auto-antistoffen gericht tegen de AIS kunnen toekomstige studies vergemakkelijken die gericht zijn op het verbeteren van inzicht in de moleculaire structuur en functie van het AIS.

De behandeling van PNS schiet nog ernstig te kort. Zoals besproken in hoofdstuk 1 zijn er argumenten voor de betrokkenheid van zowel humorale als cellulaire immuun mechanismen in de pathogenese van PNS. Onze hypothese was dat verlaging van de autoantilichaam titers in een vroeg stadium de ziekte kan stabiliseren. Vervolgens hebben we voorondersteld dat eliminatie van circulerende B-lymfocyten zou kunnen leiden tot vermindering van de secretie van auto-antistoffen in de circulatie en de voortdurende antigen presentatie zou kunnen

reduceren. Rituximab is een chimeer anti-CD20 monoklonaal antilichaam dat een snelle en langdurige eliminatie van de circulerende B ce1len veroorzaakt. Rituximab is goedgekeurd als monotherapie of met andere medicijnen voor de behandeling van bepaalde vormen van CD20 positieve B ce1 non-Hodgkin lymfomen en chronische lymfatische leukemie. Het is ook goedgekeurd voor de behandeling van refractaire reumatoïde artritis. **Hoofdstuk 6** beschrijft onze klinische trial met Rituximab in negen patiënten met een PNS geassocieerd met een hoge titer antineuronale antistoffen (acht met anti-Hu en één met anti-Yo). De anti-Yo positieve patiënte met een ovariumcarcinoom en twee van de anti-Hu positieve patiënten, beiden met een KCLC, toonden verbetering van neurologische functie. Alhoewel eliminatie of aanzienlijke vermindering van de circulerende B ce1len werd waargenomen bij alle patiënten, werd geen consistente afname van serum of liquor antilichaam titers waargenomen. Gezien de beperkte respons op immunotherapie in PNS is de verbetering van drie van de negen patiënten in deze studie suggestief voor een positief effect van Rituximab bij deze patiëntenpopulatie. Echter, de omvang van de studie was heel klein. De aanwezighied van verstorende factoren, zoals gelijktijdige cytotoxische therapie, bemoeilijkt een duidelijke conclusie over de effectiviteit van Rituximab in deze patient populatie nog verder. Om de blootstelling van het centrale zenuwstelsel te verhogen kan in de toekomst ook nog intrathecale toediening van Rituximab worden overwogen.

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DANKWOORD

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CURRICULUM VITAE

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The author is born and completed her medical education in Tehran, Iran and has been living and working last 11 years in The Netherlands. After graduation from high school in science branch, she entered the medical school of National University of Iran (Shaid Beheshty, Tehran) and received her medical degree in 1989, followed by specialization in internal medicine from the same university in 1993 and shortly after that obtained approval of her PhD thesis "Cancers of Head and Neck /Nasopharynx" with distinction. She worked as an assistant professor, teaching medicine, including oncology and in the mean time, run her private practice and working for several hospitals at the level of consultant, until late 1999 that immigrated to The Netherlands, starting with a fundamental scientific research, first as a guest researcher and then from May 2000, as a PhD candidate, in the Neuro-Oncology lab of Erasmus Medical University, Rotterdam. As due to immigration limitations, she was not able to stay longer at university to conclude her Western PhD thesis, she left Erasmus university on June 2004 to immediately start a new carrier in clinical research , working for an oncology specialized international clinical research organization and cancer research foundation, NDDO oncology and INC research. In 2007 she left the position of head of medical oncology and pharmacovigilance, Europe, to participate in first hand cancer clinical research with joining a pharmaceutical company, Astellas Pharma Global Development, as a senior medical expert which continues to date.

LIST OF PUBLICATIONS

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