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A large screen for paraneoplastic neurological autoantibodies; diagnosis and predictive values

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ABSTRACT

Background: Paraneoplastic neurological syndromes (PNS) are a group of syndromes that affect the central and peripheral neuromuscular system in association with cancer. Specific antibodies may assist in the diagnosis of PNS. The antibodies tested can be classified into those directed against intracellular neuronal proteins ("well characterized" PNS: Hu, Yo, RI, CV2, amphiphysin, Ma1, Ma2) and those directed against neural surface antigens (autoimmune encephalitis syndromes: NMDA, AMPA, LGI1, CASPR2, GABAR). We aimed to characterize patients with unexplained neuropsychiatric symptoms, in whom positive PNS antibodies were detected in the Sheba medical center, a large referral hospital.

Methods: Clinical and demographic data of patients with positive PNS antibodies were collected during the years 2002–2016. Antibodies were tested by either Line immunoassay or by cell-based indirect immunofluorscent assay.

Results: During the follow up of 14 years, 4010 PNS tests were performed in patients with unexplained neuropsychiatric symptoms. Seventy-two were found to be positive; among them we had full clinical data access to 44. The most frequent antibodies were anti-Hu (31.8%), anti-Yo (18.2%), anti-CV2 (13.6%), and anti-NMDA (9.1%), and the most common cancers were small-cell lung (SCLC) and ovarian cancers. In the well characterized paraneoplastic group, cancer was diagnosed in 55.9% of the patients, and in the autoimmune encephalitis group, 40.0% were diagnosed with cancer. A positive correlation between antibody titer and the presence of cancer was found. Ninety percent of the tests in patients who were found positive were ordered by a neurologist or neuro-oncologist.

Conclusions: The titers of PNS auto-antibodies can predict cancer in patients whom anti-PNS antibodies are tested. In addition, consultation with a specialist should be considered before this test is ordered.

1. Introduction

Paraneoplastic neurological syndromes (PNS) are a heterogeneous group of neurological syndromes. Symptoms may involve any part of the central and peripheral nervous system, the neuromuscular junction, or the muscles themselves [1]. PNS are rare and neurologic symptoms and cancer association vary [2]. For unknown reasons, the most common tumor type associated with PNS is small-cell lung cancer (SCLC) [2–4], whereas the more common clinical symptoms are Lambert-Eaton myasthenic syndrome (LEMS), sensory neuropathies, cerebellar degeneration, and limbic encephalitis [2,3]. Specific auto-antibodies are detected in the sera or cerebrospinal fluid (CSF) of PNS patients [5]. PNS patients have been classified into two groups according to the location of the antigen [6]. In the first group, antibodies

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are directed against intracellular neuronal proteins: anti-HU (a family of four RNA-binding proteins: HuD, HuC/ple21, Hel−N1, and Hel−N2, that are expressed in neurons), anti-Yo (Purkinje cell cytoplasmic antibody type 1 (PCA-1)–IgG), anti-RI (directed against an RNA binding site), anti-amphiphysin, anti-Ma2 and anti-Ma1 (antibodies directed against a 37 and 40 kDa neuronal proteins), and anti-CV2 (antibodies directed to a 66-kd protein expressed in brainstem, spinal cord, and cerebellum), and can be detected in the serum and CSF [7]. These antibodies have a strong association with the underlying tumor and are termed "well characterized" paraneoplastic or onconeuronal antibodies. The pathogenicity of the "well characterized" paraneoplastic antibodies is believed to be mediated by cytotoxic T cells, and the antibodies are thought to be just epiphenomena in most cases. In the autoimmune encephalitis group, the antibodies are directed against neuronal cell surface or synaptic proteins including receptors: anti-NMDA (Anti-*N*-methyl-D-aspartate receptor), anti-AMPA (Anti-Glutamate Receptor), anti-LGI1 (Leucine-rich, glioma inactivated 1), anti CASPR2 (anti–contactin-associated protein-like 2) and can also be detected in the serum and in the CSF. Patients with these auto-antibodies mainly suffer from autoimmune encephalitis, however overlap syndromes that affect other parts of the central nervous system may also occur [8]. The "autoimmune encephalitis" may affect children, and detection of the autoantibodies does not necessarily indicate that the disorder is neoplastic [1,6,9]. Studies in animal models and patients [10,11], the clinical response of autoimmune encephalitis to immunotherapy, and the correlation between antibody titers and neurological outcomes, suggest that the autoimmune encephalitis paraneoplastic antibodies have a direct pathogenic effect on the target antigens [6,8,12,13]. Although the detection of PNS antibodies has been useful to indicate a neurological syndrome as a paraneoplastic one, PNS symptoms can occur without PNS antibodies [4,14], and the antibodies can be found without a neurological syndrome. In addition, in some of the cases specific PNS antibodies are detected before, during, or years after the diagnosis of a neurological syndrome [2,4,15].

In the current study we present our experience in PNS antibody testing at a large tertiary medical center (Sheba medical center, Tel Hashomer, Israel) during the years 2002 to 2016 and describe the 44 patients who were found positive.

2. Materials and methods

2.1. Study design

In this retrospective study, we identified all patients in which their sera were tested for PNS antibodies at the Zabludovic Center for Autoimmune Diseases in Tel Hashomer, Israel. We gathered demographic (age, gender), neurologic- and cancer-related clinical data, as well as laboratory data from the medical records and the hospital database. The study received approval of the hospital's ethical committee. During the years 2002–2016, 4010 antibody tests were performed, among which 72 were positive. Clinical data were available for 45 of these patients.

3. Antibody testing

3.1. Antibody testing

3.1.1. Well characterized paraneoplastic antibodies

Antibodies were detected using a recombinant Line Assay (Ravo Diagnostika GmbH, Freiburg, Germany). Briefly, nitrocellulose strips, coated with recombinant antigens Hu, Yo, RI, CV2, amphiphysin, Ma1, and Ma2 were incubated with patient sera. After the extensive (5 times) washing, the strips were incubated with alkaline phosphate conjugated anti-human IgG antibodies. Bound antibodies were detected by using BCIP/NBT as a substrate. The results (qualitative) are expressed as positive, weak, and very weak based on intensity of the bands stainings.

3.1.2. Autoimmune encephalitis antibodies

In this study, we used a cell-based indirect immunofluorscent assay (CBA) with transfected HEK293 (Autoimmune Encephalitis Mosaic1 Euroimmun, Lubeck; Germany) enabled antibody detection to different neuronal (NMDA, CASPAR2, AMPA1,AMPA2 LGI1,GABAB) antigens. The results are expressed as positive, weak, and very weak based on immunofluorescence intensity of the endpoint titration of the sera samples.

3.1.3. Statistical analysis

Means, standard deviations, frequencies, and percentages were determined using Microsoft Office Excel 2007 software.

4. Results

4.1. Patient characteristic

Over a 14 years period, 4010 PNS tests for well characterized and autoimmune encephalitis autoantibodies were performed for patients undergoing workup for neuropsychiatric symptoms without an alternative explanation. Among the 4010 PNS autoantibody tests performed, 72 were found positive; 28 were excluded from the analysis due to lack of available medical records. Among 44 patients with PNS autoantibodies, the majority were women 29 (65.9%), and the mean age was 57.1 ± 16.6. Ninety percent of the tests in patients who were found positive were ordered by a neurologist or neuro-oncologist.

Thirty-four patients (77.3% of total) were positive for well characterized autoantibodies (Table 1), the most common being anti-Hu (41.2% of the well characterized PNS group), anti-Yo (23.5%), and anti-CV2 (17.6%). Four patients had two or more positive PNS autoantibodies. During the follow-up, cancer was diagnosed in 19 (55.9%) of the well characterized PNS patients. The most common cancers were small cell lung (20.0%) and ovarian cancers (6.7%). All patients presented with neurological symptoms, the most common being neuropathies (both sensory and sensory-motor neuropathies, 31.43%), cerebellar ataxia (28.57%), encephalitis (14.29%), and seizures (11.43%).

Ten patients (22.7% of total) were positive for autoimmune encephalitis PNS group antibodies (Table 1), the most common being anti-NMDA (40%) and anti-LGI1 (20%). Cancer was diagnosed in four (40%) patients. Two of these patients had malignant thymoma and two had SCLC. Neurological symptoms included encephalitis (30%), chorea (20%), cognitive impairment (20%), Lambert-Eaton myasthenic syndrome (20%), seizures (20%), and myasthenia gravis (10%). During the follow-up, 10 patients from the well characterized PNS group and one in the autoimmune encephalitis group died (seven related to cancer and four due to unknown causes). Patients were treated with chemotherapy ($n = 16$), plasmapheresis ($n = 15$), intravenous immunoglobulins (IVIG) ($n = 8$), corticosteroids ($n = 18$), tumor resection ($n = 6$), and radiation therapy ($n = 3$). The patients had positive (n = 18, 40.9%), weak ($n = 24$, 54.5%), or very weak (n = 2, 4.5%) autoantibody titers. Cancer diagnosis correlated with antibody titer: of 18 patients who tested *positive* for autoantibodies, 14 (77.8%) were diagnosed with cancer; whereas 9 of 24 (37.5%) with *weak positive* and neither of the two with *very weak positive* results developed cancer. In 10 patients, the cancer was diagnosed an average of 7.6 months before the neurological symptoms, in 12 patients the neurological symptoms appeared 36.6 months on average before the cancer. In one patient, the diagnosis of cancer was determined simultaneously.

4.2. Well characterized paraneoplastic neurological autoantibodies

In the sections below, the characteristics of the 34 patients with well characterized PNS who tested positive for particular antibodies are discussed. The patient characteristics, neurological symptoms, cancer diagnoses, and treatment data are summarized in Table 2.

Table 1
Paraneoplastic antibodies and cancer associations in PNS cohort.

Group	Antibody	N (%)	Avg. age	F:M	Cancer association	Neurological symptoms
1: Well characterized antibodies (n = 34)	Anti-HU	14 (31.8%)	54.5 ± 17.8	12:2	SCLC (50%) CLL (7.1%) RCC (7.1%) Plasmocytoma (7.1%) No cancer (28.6%)	Ataxia Limbic encephalitis Sensory neuropathy Sensory-motor neuropathy
	Anti-YO	8 (18.2%)	65.3 ± 12.2	6:3	Ovarian cancer (37.5%) Breast cancer (12.5%) Colon cancer (12.5%) Melanoma (12.5%) No cancer (25%)	Ataxia Limbic encephalitis Sensory-motor neuropathy
	Anti-CV2	6 (13.6%)	43.8 ± 11.2	2:4	SCLC (16.7%) Low grade lymphoma (16.7%) MM (16.7%) No cancer (66.7)	Sensory neuropathy Sensory-motor neuropathy Ataxia CIDP Nystagmus Epilepsy MS
	Anti-Ma2	3 (6.8%)	49 ± 20.4	3:0	SCLC (33.3%) No cancer (66.6%)	Cerebellar ataxia Extrapyramidal signs MS Seizures
	Anti-RI	2 (4.5%)	65 ± 14.1	2:0	No cancer (100%)	Sensory- motor neuropathy Psychiatric disorder
	Anti- amphiphysin	1 (2.3%)	68	0:1	No cancer (100%)	Cerebellar ataxia
2: Autoimmune encephalitis antibodies (n = 10)	Anti-NMDA	4 (9.1%)	22.5 ± 26.0	3:1	No cancer (100%)	Chorea Limbic encephalitis Ataxia Opsoclonus Paraplegia
	Anti-LGI1 (previously anti-VGPC)	2 (4.5%)	64.5 ± 4.9	0:2	No cancer (100%)	Seizures Limbic encephalitis Memory loss
	Anti-CASPR2 (previously anti-VGPC)	2 (4.5%)	39.5 ± 3.5	0:2	Thymoma (100%)	MG Morvan syndrome Visual hallucinations
	Anti-VGCC	2 (4.5%)	53 ± 2.8	2:0	SCLC (100%) Cervical cancer (50%)	LEMS
	Total	44	57.1 ± 16.6	29:15		

Abbreviations: PNS – paraneoplastic syndrome; SCLC – small cell lung cancer; CLL – chronic lymphocytic leukemia; RCC – renal cell carcinoma; MM – multiple myeloma; CIDP – chronic inflammatory demyelinating polyneuropathy; MS – multiple sclerosis; NMDA – *N*-methyl D-aspartate; LGI1 – leucine-rich, glioma inactivated 1; CASPR2 – contactin associated protein-2; MG – myasthenia gravis; VGCC – voltage-gated calcium channel; LEMS – Lambert-Eaton myasthenic syndrome.

4.2.1. Anti-Hu

Antibodies to Hu were the most frequently detected autoantibodies in the 34 patients with well characterized PNS antibodies (N = 14; 41.2% of well characterized PNS group). The majority of the patients with anti-Hu antibodies were female (85.7%). The most frequent associated cancer by far was SCLC (50%), and no cancer was detected in 28.6% of patients. All patients suffered from neurological symptoms: mainly cerebellar ataxia, limbic encephalitis, sensory and sensory-motor neuropathies. In those patients in whom the cancer diagnosis preceded presentation of the neurological symptoms, the time between diagnoses ranged from 1 to 12 months. Six patients have died in this group during the follow up period (3 from cancer and 3 due to unknown cause) (Table 3).

4.2.2. Anti-Yo

Anti-Yo antibodies were detected in eight patients; five were female. Cancer was diagnosed in 6 patients, the most common being ovarian cancer (n = 3). The most frequent neurological symptoms were ataxia, encephalitis, and sensory-motor neuropathy. The duration between the cancer diagnosis and the presentation of the neurological symptoms was up to 1 year, except in one case in which the cancer preceded the neurological symptoms by 5 years. Three patients have died in this group during the follow up time (2 from cancer and 1 due to unknown cause).

4.2.3. Anti-CV2

Anti-CV2 antibodies were detected in six patients. The dominant sex was male (66.7%). No tumors were found in 66.7% of patients. The neurological symptoms entailed neuropathies of different types, predominantly sensory and chronic inflammatory demyelinating polyneuropathy (CIDP)-like, ataxia, nystagmus, epilepsy, and demyelination. One patient in this group died due to cancer during the follow up.

4.2.4. Anti-Ma2

Antibodies to Ma2 were detected in three of the cases. One patient had SCLC and two did not have cancer. The neurological symptoms were cerebellar ataxia, extrapyramidal signs, demyelination, and seizures. The patients carried weak or very weakly positive anti-Ma1 antibodies.

4.2.5. Anti-RI

Anti-RI antibodies were detected in two cases; both were female. No cancer was found in these patients. The neurological symptoms were sensory- motor neuropathy in one patient, and a psychiatric disorder in the other.

4.2.6. Anti-amphiphysin

Antibody to amphiphysin was found in one male patient. No cancer was reviled, and the neurological symptom was cerebellar ataxia.

Table 2
Autoantibody expression and clinical data of patients with well characterized PNS antibodies.

No	Gender	Age	Symptoms	Titer	Imaging	Cancer	Tx	Presenting[a]	D[b]
Anti-Hu antibodies									
1	F	57	Limbic encephalitis Cerebellar degeneration	Positive	Multiple sub-cortical brain signals	SCLC	PP GC Chemo	PNS (2 m)	
2	F	65	Sensory motor-neuropathy Confusional state	Positive	White matter and brain stem signals	SCLC	IVIG GC PP Chemo	Cancer (12 m)	
3	F	51	Cerebellar ataxia Dysarthria	Positive	Normal brain MRI	SCLC	PP GC Chemo Rad	Cancer (6 m)	
4	F	57	Sensory motor-neuropathy	Positive	Normal brain CT	SCLC	PP GC Chemo	PNS (1 m)	D
5	F	47	Limbic encephalitis Epilepsy	Weak	Normal brain MRI	Plasma-cytoma	IVIG GC RTX Rad VNS	PNS (12 m)	
6	F	74	LEMS, vision impairment	Positive	Normal brain CT	SCLC	Chemo	PNS (2 m)	D
7	M	80	Mild sensory neuropathy	Weak	NA	SCLC/NET	Chemo	Cancer (2 m)	D
8	F	49	Ataxic sensory neuropathy	Weak	NA		Gabapentin		
9	F	18	Epilepsy	Weak	Normal brain MRI				
10	F	81	CIDP	Weak	NA	CLL	Chemo	Cancer (7 y)	D
11	F	23	Limbic encephalitis	Weak	Normal brain CT				
12	M	68	Sensory motor-neuropathy Limbic encephalitis	Hu-positive RI-positive	Normal brain CT				D
13	F	58	Sensory motor-neuropathy Ataxia	Hu-positive CV2-weak	Normal brain CT	SCLC	PP GC IVIG Chemo	PNS (3 m)	
14	F	62	Left leg weakness	Hu-positive CV2 -weak	Spinal metastasis	RCC	Surgery	Cancer (2 m)	D
Anti-Yo antibodies									
1	F	80	Pyramidal signs	Weak	NA	Breast	PK Mertz Dopicar	Cancer (14 y)	
2	F	45	Dizziness Diplopia Ataxia Rotatory nystagmus	Positive	MRI – Lepto-meningeal spread	Ovarian	Surgery Chemo GC	Cancer (1 y)	D
3	M	60	OCD with psychotic features	Weak	MRI – Periventricular white matter lesions				
4	F	73	Cerebellar ataxia Cognitive dysfunction	Positive	MRI – Cerebellar atrophy	Ovarian	GC PP	PNS (1 y)	D
5	F	53	CN 7,6 Palsy Limbic encephalitis	Positive	MRI –Leptomeningeal spread	Ovarian	GC WBRT Chemo Surgery	Cancer (5 y)	D
6	M	71	Sensory-motor distal axonal polyneuropathy	Weak	NA				
7	M	80	Acute dementia	Weak	CT – Basal ganglia calcification	Melanoma	Surgery	Cancer (6 m)	
8	F	57	Cerebellar ataxia Rotatory nystagmus	Yo –Weak CV2 –Weak	CT – Normal	CRC	Chemo Surgery	Cancer (1 y)	
Anti-CV2 antibodies									
1	M	43	CIDP	Weak	CT – Normal	Low-grade lymphoma MM	GC Chemo BMT IVIG	PNS (1 y)	
2	F	55	Sensory neuropathy	Weak	MRI – Non-enhanced periventricular lesions				
3	M	55	Sensory neuropathy	Weak	CT – Normal	SCLC	Chemo Rad GC	Cancer (9 m)	D
4	M	69	Sensory-motor neuropathy	Weak	NA		IVIG		
5	F	30	Epilepsy MS	Weak	MRI – Diffuse demiyelinating disease		IVIG		

(*continued on next page*)

Table 2 (*continued*)

No	Gender	Age	Symptoms	Titer	Imaging	Cancer	Tx	Presenting[a]	D[b]
6	M	36	Cerebellar ataxia Opsoclonus Myoclonus	Weak	MRI – Normal		PP		
Anti-MA2 antibodies									
1	F	56	Cerebellar ataxia Extrapyramidal signs	Weak	MRI – Cerebellar and vermis atrophy		PP IVIG		
2	F	26	MS	Weak	NA				
3	F	65	Seizures	Weak	CT – Brain metastasis	SCLC	Chemo WBRT GC	Cancer (0 m)	
Anti-RI antibodies									
1	F	55	Psychiatric disorder	Weak	NA		GC MTX		
2	F	75	Sensory- motor neuropathy	Very weak	CT – Old infarcts		IVIG		
Anti-amphiphysin									
1	M	68	Cerebellar ataxia	Weak	MRI – Atrophy				

Abbreviations: PNS – paraneoplastic syndrome; Tx – treatment; F – female; SCLC – small cell lung cancer; PP – plasmapheresis; GC – glucocorticoids; chemo – chemotherapy; IVIG – intravenous immunoglobulins; MRI - magnetic resonance imaging; Rad – irradiation therapy; RTX – rituximab; VNS – Vincristine, LEMS – Lambert-Eaton myasthenic syndrome; CT – computed tomography; M – male; NET – neuroendocrine tumor; CIDP – chronic inflammatory demyelinating polyneuropathy; MM – multiple myeloma; RCC- renal cell carcinoma; MS – multiple sclerosis; PNS – paraneoplastic syndrome; CLL – chronic lymphocytic leukemia; GBS - Guillain–Barré syndrome; NMDA - N-methyl D-aspartate; LGI1 - Leucine-rich, glioma inactivated 1; CASPR2 - Contactin Associated Protein-2; VGCC - Voltage-gated calcium channel; BMT – bone marrow transplant; NA – not available; Ca- cancer; OCD –Obsessive–compulsive disorder; WBRT - Whole brain radiation therapy; CN – cranial nerves; MTX – methotrexate.

[a] The disease diagnosed first is given and the numbers in parentheses represent the time before diagnosis of the other disease. m indicates the number in months, y the number in years.

[b] D – meaning deceased. In this column, the letter D mentions the patients who died during follow up.

4.3. Autoimmune encephalitis autoantibodies

Ten patients had autoimmune encephalitis autoantibodies. Patient characteristics, the neurological symptoms, the cancer diagnoses, and treatment data are summarized in Table 2.

4.3.1. Anti-NMDA receptor

Antibodies to the NMDA receptor were reported in four patients (40% of those with autoimmune encephalitis antibodies). Of those with anti-NMDA receptor antibodies, 3 were female. The ages of these patients varied widely: 1, 2, and 55 years old for the females and 32 for the male patient. No cancer was found in this group. Neurological symptoms entailed chorea, limbic encephalitis, ataxia, opsoclonus, and paraplegia.

4.3.2. Anti-LG1

The antigen of this autoantibody was previously known as a voltage-gated potassium channel (VGPC). The anti-VGPC antibodies have been classified into two groups: anti-leucine-rich glioma inactivated protein 1 (LG1) and anti contactin-associated protein-2 (anti-CASPR2). Anti-LGI1 antibodies were detected in two patients (20%). None of them were diagnosed with cancer. The neurological presentations were seizures, memory loss, and encephalitis.

4.3.3. Anti-CASPR2

This antibody targets contactin-associated protein-2. In our cohort, anti-CASPR2 antibodies were detected in two patients (20% of the autoimmune encephalitis antibody group). Both of the patients were male (average age 39.5). Both were diagnosed with malignant thymoma prior to the onset of the neurological symptoms. The symptoms appeared one and five years after thymectomy. The neurological presentations were myasthenia gravis (MG), Morvan's syndrome, and visual hallucinations.

4.3.4. Anti-VGCC

Anti- voltage gated calcium channel (VGCC) antibodies were detected in two patients (20% in the autoimmune encephalitis antibody group). Both patients were female, and were diagnosed with Lambert-Eaton myasthenic syndrome. Both patients had SCLC, and one of the patients also had a cervical cancer. The duration between cancer diagnosis and presentation of the neurological symptoms was 2 months in one patient and 2 years in the second patient. One patient died from cancer during the follow-up period.

5. Discussion

During the years 2002–2016, our center performed 4010 PNS autoantibody tests. We describe 44 PNS patients, as categorized previously, into well characterized (n = 34) and autoimmune encephalitis (n = 10) PNS types [1,16]. The majority of the patients were females (65.9%), with the exceptions of the patients with anti-LGI1, anti-CASPR2 and anti-amphiphysin antibodies, who were predominantly males. Cancer was diagnosed in 52.3% of all patients, and nearly half of the patients with cancer were diagnosed with SCLC. While SCLC accounts for only 15% of the lung cancers, for unknown reasons it has the highest rate of PNS reported. In a recent study, 9.4% of SCLC patients were diagnosed with PNS; however in contrast to our study, in that study, the PNS antibody panel included a newer autoantibody, the anti-SOX2 [17] which is useful in detecting cancers. Ovarian cancer, associated with anti-Yo autoantibodies was the second most frequently diagnosed cancer in our cohort.

In concordance with previous studies, patients with 'well characterized' PNS antibodies were more likely to be diagnosed with cancer than those with the autoimmune encephalitis autoantibodies [6]. In addition, our analysis demonstrated that higher semi-quantification titers of PNS antibodies correlated well with a cancer diagnosis. Previous studies suggested that the presences of low PNS antibody titers are associated with less cancer diagnoses as well as better overall outcomes [18,19]. High antibody titers also correlated with the presence of

Table 3
Autoantibody expression and characteristics of patients with autoimmune encephalitis antibodies.

Anti-NMDA receptor antibodies

No	Gender	Age	Symptoms	Titer	Imaging	Cancer	Tx	Presenting[a]
1	M	32	Confusion	Weak				
2	F	1	Chorea Opsoclonus	Very weak	MRI – Small hyper intense putamens bilaterally			
3	F	55	Dementia paraplegia	Positive	MRI – Normal		PP GC	
4	F	2	Ataxia Chorea Encephalitis	Positive	MRI – Normal		GC IVIG PP RTX	

Anti-LGI1

No	Gender	Age	Symptoms	Titer	Imaging	Cancer	Tx	Presenting[a]
1	M	68	Encephalitis Short-term amnesia Seizures	Positive	MRI – Normal	None	IVIG GC PP AZA	
2	M	61	Seizures	Weak		None	GC	

Anti -CASPR2 antibodies:

No	Gender	Age	Symptoms	Titer	Imaging	Cancer	Tx	Presenting[a]
1	M	42	MG	Positive	MRI – Left prechiasmatic fullness	Malignant thymoma	Chemo Surgery RTX GC PP	Cancer (1 y)
2	M	37	Fluctuative hallucinations Morvan syndrome	Positive		Malignant thymoma	PP Surgery	Cancer (5 y)

Anti-VGCC antibodies:

No	Gender	Age	Symptoms	Titer	Imaging	Cancer	Tx	Presenting[a]
1	F	55	LEMS	Positive		SCLC	PP	PNS (2 m)
2 d	F	51	LEMS	VGCC – Positive Hu – Positive	MRI - normal	SCLC Cervical CA	PP AZA Firdapse	PNS (2 y)

Abbreviations - SCLC – small cell lung cancer; MG – myasthenia gravis; MM – multiple myeloma; LEMS – lambert eaton myasthenic syndrome; RCC- renal cell carcinoma; MG – myasthenia gravis; MS – multiple sclerosis; Pns – paraneoplastic syndrome; CLL – chronic lymphocytic leukemia; CIDP - Chronic Inflammatory Demyelinating Polyneuropathy; NMDA - N-methyl D-aspartate; LGI1 - Leucine-rich, glioma inactivated 1; CASPR2 - Contactin Associated Protein-2; VGCC - Voltage-gated calcium channel; BMT – bone marrow transplant; Pos – positive; PP – plasmapheresis; GC – glucocorticoids; chem. – chemotherapy; IVIG – intravenous immunoglobulins; Rad – irradiation therapy; CT – computed tomography; RTX – rituximab; NET – neuroendocrine tumor; NA – not available; M – male; F- female; Ca- cancer; OCD –Obsessive–compulsive disorder; MRI - Magnetic resonance imaging; WBRT - Whole brain radiation therapy; CN – cranial nerves; MTX – methotrexate; RTX – rituximab; AZA – azathioprone; LT – left;

[a] The right column represents the first manifestation: whether it being cancer or paeaneoplastic syndrome (PNS), The numbers in the brackets represent the time gap between presentations. The letters - m represents the numbers in months, y represents the numbers in years.

the neurological symptoms in cancer patients. For example, high titers of anti-Hu antibodies in patients with SCLC are associated with PNS, whereas patients with SCLC and with lower titers of anti-Hu antibodies do not have neurologic symptoms [20]. The presence of low titers of paraneoplastic antibodies in cancer patients without neurological symptoms led to the idea that these antibodies can be used in some cases as a biomarker for the early detection of cancer [21–23]. For example, one study found 11 positive onconeuonal antibodies (7 anti-Ri, 4 anti-Yo), among 180 patients with ovarian cancer, which were not associated with neuropsychiatric symptoms [24]. It has been suggested that the level of autoantibody titers, such as anti-Hu, correlate also with the differentiation of neoplasm: in another study, tumors that were poorly differentiated were associated with higher titers, whereas patients without antibodies had well differentiated tumors [18]. We did not find a correlation between the antibodies titers and tumor differentiation in our study. A connection between titers and clinical outcomes was shown before also in patients with autoimmune encephalitis antibodies. According to the work of Petit-Pedrol et al., anti-GABA titers are present in patients with more severe disease, and low titers are associated with CSF negativity and less specific clinical presentation, corresponding with a broader spectrum of symptoms [25]. Neurologic improvement has also been correlated with a decrease in anti-GABA titers [26,27]. Other studies however have indicated that follow-up of serum antibody titers is an unreliable biomarker of disease activity and should not be used as the main guide for clinical decisions [28]. In our cohort, we detected more than one paraneoplastic antibody in four

patients. Pittock et al. suggested that multiple autoantigens may be predictive of specific cancers [29], but we could not identify a trend in our patients for the neurological symptoms or cancer types.

Most of the patients with well characterized PNS antibodies were elderly; none were under the age of 18. Patients with autoimmune encephalitis antibodies were of all ages. Anti-NMDA autoantibodies were detected in three children aged 1, 2, and 6 years old. Indeed, in previous cohorts, patients with autoimmune encephalitis antibodies were younger, more responsive to immunotherapy, and less likely to have tumors than were patients with well characterized PNS antibodies [30,31]. Interestingly in has been demonstrated that SLE and SS patients with neuropsychiatric syndromes have elevated levels of NR2A/B antibodies, a subunit of Anti- NMDA [32].

Fourteen of our patients had anti-Hu PNS. The majority were female, and the common manifestations were sensory-motor peripheral neuropathy (28%), encephalitis (28%), and cerebellar ataxia (21%). In contrast, in the group of 200 patients described by Graus et al. with anti-Hu PNS [33], 75% were male and common neurological presentations were sensory neuropathy (54%), cerebellar ataxia (10%), and encephalitis (9%). We could not find an explanation for those differences. Among our eight patients with anti-Yo PNS, 50% were diagnosed with ovarian or breast malignancies. Ataxia, encephalitis, and sensory-motor neuropathy were the most commonly observed symptoms. Cerebellar dysfunction with breast or ovarian cancers were previously reported to predominate among anti-Yo associated PNS patients [15,34]. Interestingly, cerebellar degeneration-related antigens CDR2 and CDR2L, which were found to be the targets for anti-Yo antibodies, are expressed mainly on ovarian cancer tissues [35–37]. It should be noted that the clinical utility and relevance of anti-Yo as a screening test in cancer patients is difficult to determine. Monstad et al. showed that the prevalence of anti-Yo was 2.3% in 557 ovarian cancer patients and 1.6% in 253 breast cancer patients, and that only two (11.8%) of all the patients with anti-Yo had PNS [38].

The main limitation of this study is that out of 72 patients, we did not have clinical information regarding 28 of the patients. This might have had an impact on the rates of neurological symptoms and cancers.

6. Conclusions

The PNS are a rare, yet rising entity, in which large sources of information are lacking currently. In our study we included a relatively large number of patients with PNS antibodies, among them we were able to analyze the symptoms and underline disease in 44 patients. Clinicians should be aware of the paraneoplastic neurological syndromes in order to identify patients in which neurological signs and symptoms may warrant cancer work-up. In addition, a minority of patients suffering from psychiatric manifestations may be found to have autoimmune limbic encephalitis, in which immune-suppression is an effective treatment. In our center, the majority of positive PNS tests were ordered by a neurologist or neuro-oncologist. This is important due to the considerable high cost of the test, and specialists should be consulted before this test is ordered.

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