Supplementary Online Content

Martinez-Hernandez E, Ariño H, McKeon A, et al. Clinical and immunologic investigations in patients with stiff-person spectrum disorders. *JAMA Neurol.* Published online April 11, 2016. doi:10.1001/jamaneurol.2016.0133.

eMaterial.

This supplementary material has been provided by the authors to give readers additional information about their work.

eMaterial

Patients excluded after identifying a final diagnosis other than SPSD

Patients with a final diagnosis of other disorders included, 6 cases with a functional disorder, 4 peripheral nerve hyperexcitability, 2 sporadic Creutzfeldt-Jacob disease, 2 amyotrophic lateral sclerosis, and one of each, tetanus, subacute combined spinal cord degeneration, primary progressive multiple sclerosis, Hashimoto encephalopathy, myotonia congenita, and myopathy.

Patients with immune mediated CNS disorders used as controls

Among the 195 patients with other immune mediated CNS disorders used as controls, 75 (44 with paired CSF) had GAD65 antibodies and symptoms other than SPS (cerebellar ataxia, epilepsy, or limbic encephalitis); 55 (30 with CSF) multiple sclerosis; 55 (49 CSF) encephalitis associated to neuronal-surface antibodies (NMDA-receptor, LGI1, or GABAb-receptor), and 10 anti-Hu associated paraneoplastic symptoms.

Antibody assays

Serum and CSF were examined for GAD65 and amphiphysin antibodies using frozen sections of paraformaldehyde (PFA) perfused sagittal sections of rat brain, as reported.[1] The presence of GAD65 antibodies was confirmed with cell-based assay (CBA) as described,[2] and amphiphysin antibodies were confirmed with immunoblot. GlyR and GABAaR antibodies were determined with live CBAs as reported.[3, 4] Antibodies to gephyrin were determined by CBA using HEK293 cells expressing (transfection ratio 1:1) cherry-labeled human Gephyrin and hPEM2 or collybistin, a protein necessary for membrane anchorage of gephyrin. HEK293 cells were fixed for 10 minutes in 4% PFA, permeabilized, and incubated with patients' serum (1:40) or CSF (1:5) overnight at 4ºC, followed by the secondary Alexa Fluor 488 goat anti-human antibody (1:1000, A11013 Molecular

Probes/ Life Technologies). Antibodies to the Glycine transporters 1 and 2 (GlyT1, GlyT2) were determined using HEK293 cells expressing myc-tagged human GlyT1 or GlyT2. Cells were incubated with patients' serum (1:40) or CSF (1:5) for 1 hour at 37ºC, fixed and permeabilized, and serially incubated with a myc-tag mouse monoclonal antibody (1:5000, 2276, Cell Signaling Technology, Inc.), the indicated anti-human secondary antibody, and Alexa Fluor 594 goat anti-mouse (11005, Molecular Probes/Life Technologies).

Additional antibodies against neuronal cell surface proteins were determined in serum and CSF using an immunofluorescence assay with dissociated rat hippocampal neuronal cultures as reported.[5] Positive cases were tested for antibodies to DPPX or other antigens (NMDAR, AMPAR, GABAbR, Caspr2, LGI1) by CBAs, as reported.[6] Results were photographed under fluorescence microscopy (Zeiss Axioimager M2) using Zeiss Axiovision software (Zeiss, Oberkochen, Germany).

Summary of 5 patients with antibodies other than GAD65 or GlyR

Five patients without GAD65 and GlyR antibodies had autoantibodies against other known antigens (2 amphiphysin, 2 GABAaR, and 1 DPPX). One of the two patients with amphiphysin antibodies had SPS with limbic encephalitis and breast cancer, and the other had SLS in association with breast cancer. The patient with DPPX antibodies developed SPS along with hyperekplexia, myoclonus, upper motor neuron dysfunction, sensory symptoms, orthostatic hypotension and prominent pruritus, all symptoms preceded by diarrhea and 15% weight loss. The 4 patients with GABAaR antibodies (2 of them with concurrent GAD65 antibodies) were young male (median 20 years, range 12-46) who developed classic SPS (n=2), SLS (n=1), and overlapping SPS with epilepsy (n=1, discussed above); these cases have been previously reported.[4]

eReferences

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5. Lancaster E, Lai M, Peng X, et al. Antibodies to the GABAB receptor in limbic encephalitis with seizures: case series and characterisation of the antigen. *Lancet Neurol*. 2010;9(1):67-76.

6. Boronat A, Gelfand JM, Gresa-Arribas N, et al. Encephalitis and antibodies to dipeptidyl-peptidase-like protein-6, a subunit of Kv4.2 potassium channels. *Ann Neurol*. 2013;73(1):120-128.

eTable 1. Clinical Features According to the Type of Syndrome

Clinical features	SPS N=50 (41%)	SLS N=24 (20%)	SPS-plus N=37 (31%)	Overlapping syndromes N=10 (8%)	p value
Female sex (%)	30 (60)	18 (75)	20 (54)	7 (70)	0.38
Age at onset, median years (IQR)	47 (38-56)	47 (45-58)	60 (44-66)	52 (28-63)	0.055
Delay to diagnosis, median years (IQR)	2 (1-5)	1 (0-3)	1 (0-2.5)	3 (0.5-5)	0.151
Systemic autoimmune disorders	26 (52)	13 (54)	13 (35)	4 (40)	0.51
Cancer	0 (0)	1 (4)	1 (3)	1 (10)	0.49
CMUA on EMG	21/32 (65.6)	10/19 (53)	17/28 (61)	4/5 (80)	0.49
CSF pleocytosis	4/24 (17)	0/11 (0)	10/28 (36)	1/6 (17)	0.017
CSF OCBs	7/23 (30)	1/13 (8)	9/24 (38)	1/3 (33)	0.17
Antibodies:					0.002
GAD65	27 (54)	10 (42)	7 (19)	8 (80)	
GlyR[a]	5 (10)	5 (21)	12 (32)	0 (0)	
Other	1 (2)	2 (8)	1 (3)	1 (10)	
Maximum mRS score, median (IQR)	4 (2.5-4)	3 (2-4)	4 (3-5)	4 (2-5)	0.002*
Symptomatic treatment	42 (84)	16 (67)	21 (57)	5 (50)	0.006
Type of immunotherapy:					0.009
Non-treated	10 (26)	6 (32)	1 (3)	1 (13)	
First line only	19 (50)	9 (47)	10 (31)	3 (37)	
First line and long-	5 (13)	1 (5)	10 (31)	0 (0)	

term oral					
First and second line	4 (10)	2 (11)	9 (28)	4 (50)	
Other	1 (3)	1 (5)	2 (6)	0 (0)	
Cases with follow-up	34 (68)	14 (58)	29 (78)	5 (50)	0.22
Follow-up period, median months (IQR)	17 (11-66)	54 (12-72)	18 (9-42)	24 (15-37)	0.54
Relapsing course	9 (26.5)	3 (21)	12 (41)	2 (40)	0.61
Final mRS score, median (IQR)	2 (1-3)	2 (1-3)	2 (1-5)	3.5 (2-5)	0.39
Change in mRS score, median (IQR)	1 (0 - 1)	1 (0.75 - 2)	2 (-1 - 3)	0.5 (-0.75 - 1)	0.51

CMUA: continuous motor unit activity; IQR: interquartile range; mRS: modified Rankin scale; OCBs: oligoclonal bands; SLS: stiff-limb syndrome; SPS: stiff-person syndrome; SPS-plus: progressive encephalomyelitis with rigidity, myoclonus, or other symptoms.

[a] Two additional patients had concurrent GAD65 and GlyR antibodies

* Mann-Whitney U test: maximum mRS SP-plus vs SPS p=0.001, SPS-plus vs SLS p=0.002, SPS-plus vs overlapping syndromes p=1

eTable 2. Clinical Features of the Main Immunologic Groups With EMG Findings Consistent With SPS (Continuous Agonist-Antagonist Motor Unit Activity)

Clinical features	**GAD65+ N=26 (54%)**	**GlyR+ N=6[a] (13%)**	**Antibody-negative N=16 (33%)**	**p value**
Female sex (%)	23 (88)	1 (17)	8 (50)	0.001
Age at onset, median years (IQR)	54 (45-65)	52 (33-62)	47 (36-63)	0.38
Delay to diagnosis, median years (IQR)	2 (1-4)	0 (0-2)	1.5 (0.5-5)	0.10
Syndrome:				0.005
SPS	13 (50)	0 (0)	7 (44)	
SLS	7 (27)	0 (0)	2 (12)	
SPS-plus	3 (11)	6 (100)	6 (38)	
Overlapping syndromes	3 (11)	0 (0)	1 (6)	
Hyperekplexia	9 (35)	5 (83)	4 (25)	0.021
Myoclonus	2 (8)	4 (67)	3 (19)	0.014
Brainstem	2 (8)	6 (100)	2 (13)	<0.001
Pyramidal	1 (4)	4 (67)	5 (31)	0.001
Sphincter	1 (4)	3 (50)	5 (31)	0.039
Sensory	0 (0)	3 (50)	2 (13)	0.013
Autonomic	2 (8)	4 (67)	2 (13)	0.034
Insomnia	2 (8)	4 (67)	1 (6)	0.013
Systemic autoimmune disorders[b]	18 (69)	3 (50)	6 (38)	0.25
CSF pleocytosis	2/11 (18)	2/6 (33)	1/11 (9)	0.029
CSF OCBs	6/14 (42)	1/6 (17)	0/6 (0)	0.016
Maximum mRS score, median (IQR)	4 (3-4)	5 (4-5)	4 (3-5)	0.012*
Symptomatic treatment	26 (100)	4 (67)	12 (75)	0.007
Type of immunotherapy:				0.05
Non-treated	5 (19)	0 (0)	2 (13)	
First line only	12 (46)	0 (0)	6 (38)	
First line and long-term oral	4 (15)	3 (50)	2 (12.5)	
First and second line	3 (12)	3 (50)	0 (0)	
Other	1 (4)	0 (0)	1 (6)	
Cases with follow-up	25 (96)	6 (100)	11 (69)	0.034
Follow-up period, median months (IQR)	32 (11-72)	27 (9-129)	48 (12-75)	0.91
Relapsing course	11 (44)	3 (50)	5 (45)	1
Final mRS score, median (IQR)	2.5 (1-3)	1.5 (1-5)	2.5 (1.5-5)	0.68

Improvement:				0.70
Treated	16/20 (80)	4/6 (67)	5/9 (56)	
Non-treated	2/5 (40)	0 (0)	1/2 (50)	
Change in mRS score, median (IQR)	1 (0 - 2)	3 (-0.25 - 4)	0.5 (-0.25 - 2)	0.24

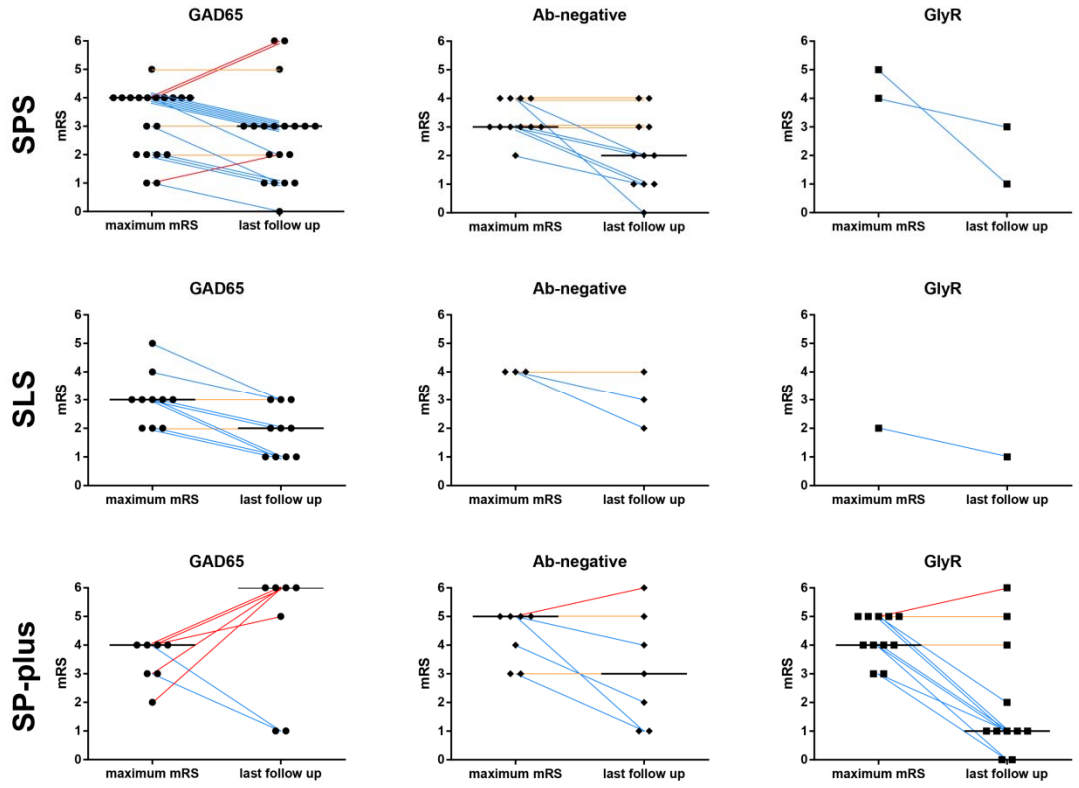
IQR: interquartile range; mRS: modified Rankin scale; SLS: stiff-limb syndrome; SPS: stiff-person syndrome; SPS-plus: progressive encephalomyelitis with rigidity, myoclonus, or other symptoms.

[a] Excludes 1 patient with concurrent GAD and GlyR antibodies

[b] 27 patients (56%): including 6 with type I diabetes mellitus, 6 thyroiditis, 1 celiac disease, 1 psoriasis, 1 vitiligo, 1 Raynaud syndrome, and 11 had thyroid, ANA, dsDNA or gastric parietal cell antibodies

* Mann-Whitney U test: maximum mRS score GAD65+ vs GlyR p=0.002, GAD65+ vs Ab-neg p=0.22, GlyR+ vs Ab-neg p=0.037

eFigure. Outcome According to the Clinical Syndrome and Immunologic Group



Patients' maximum mRS score compared with the score at the last follow-up according to the main syndromes: first row stiff-person syndrome (SPS), second row stiff-limb syndrome (SLS), and third row stiff-person syndrome plus (SPS-plus); and according to the immunologi group: first column GAD65 antibodies, second column antibody-negative (Ab-neg), and third column GlyR antibodies. Improvement for each individual patient is depicted in blue, deterioration in red, and no change in orange. The median mRS is provided as a horizontal black line (only provided if $n \geq 5$). In multivariable analysis on group level, presence of GAD65 antibodies was associated with less favorable outcome (Table 2).