Cluster Analysis of an Array of Autoantibodies in Neuropsychiatric Systemic Lupus Erythematosus

To the Editor:

Neuropsychiatric symptoms in patients with systemic lupus erythematosus (SLE) present a challenge to the clinician because they can be caused by the underlying disease (neuropsychiatric SLE; NPSLE) or coexist independently[1]. No specific diagnostic test is available for NPSLE. Reports on the associations between specific antinuclear autoantibodies and distinct NPSLE syndromes have been conflicting[2,3,4,5], perhaps because of the laboratory tests used to detect these autoantibodies. New multiplex technologies for the detection of autoantibodies have emerged in the last years and might be helpful in diagnosing NPSLE. We hypothesized that a cluster of autoantibodies could be associated with a specific NPSLE syndrome or with focal or diffuse NPSLE manifestations. Therefore we used an addressable laser bead immunoassay test in patients who visited the NPSLE clinic in Leiden, the Netherlands, a tertiary referral center for patients with SLE who have neuropsychiatric symptoms.

Between September 2007 and February 2012, 133 patients with SLE who had neuropsychiatric symptoms were evaluated and diagnosed consecutively by a multidisciplinary team[6]. All patients fulfilled the revised SLE criteria of the American College of Rheumatology (ACR)[7]. In 81 (61%) patients a diagnosis of NPSLE was established, whereas in the remaining patients the neuropsychiatric complaints were not attributed to SLE. The mean age of patients was 42.9 years (range 13–79), and 89% were female.

The serum samples of all patients were analyzed using the FIDIS connective profile kit (Theradiag), a semiquantitative homogeneous fluorescent-based microparticles immunoassay for the simultaneous detection of these autoantibodies: anti-SSA (Ro60), anti-SSB, anti-TRIM21 (Ro52), anti-Sm, anti-Sm/RNP, anti-Jo1, anti-centromere B protein, anti-ribosomal-P, anti-dsDNA, anti-histone, anti-PmScl, and anti-PCNA. Further, anticardiolipin (aCL) IgG and IgM antibodies and lupus anticoagulant (LAC) status were available from the clinical evaluation.

We performed hierarchical cluster analyses using R statistical software (version 3.0.2 for Windows) on (1) all autoantibodies from the microarray kit, and (2) all autoantibodies from the microarray kit plus the antiphospholipid antibodies (aPL), and we analyzed their associations with NPSLE diagnosis, with the ACR NPSLE syndromes, and with the groups of patients with either focal or diffuse NPSLE manifestations[8]. Statistical significance was defined as $p < 0.05$. In the first cluster analysis we identified 3 separate clusters of autoantibody profiles (no specific autoantibodies, anti-dsDNA/anti-SSA/anti-SSB/anti-TRIM21, and anti-Sm/RNP); however, no association with NPSLE diagnosis or with NPSLE syndromes was found. In the second cluster analysis, after inclusion of aPL, we identified 4 separate clusters of autoantibodies (Table 1). Three clusters identified in our analysis were similar to autoantibody profiles previously described in patients with SLE by To and Petri[9]. In our analysis we additionally identified a cluster characterized by the absence of specific autoantibodies.

The frequency of major focal syndromes was significantly higher in cluster 4 (anti-dsDNA/LAC/aCL IgM/IgG) than in other clusters ($p = 0.008$). Of the major focal syndromes, specifically cerebrovascular disease ($p = 0.030$) and seizure disorder ($p = 0.048$) were more frequent in cluster

Table 1. Sorting of 133 SLE patients with neuropsychiatric symptoms into 4 clusters by cluster analysis based on the results of a multiplex autoantibody profile and the presence of antiphospholipid antibodies/LAC.

Characteristics	Cluster 1, none, n = 23	Cluster 2, anti-dsDNA/anti-SSA/ anti-SSB/anti-TRIM21, n = 40	Cluster 3, Sm/RNP, n = 16	Cluster 4, anti-dsDNA/LAC/ aCL, n = 54	p*	p of Grouped Clusters* 1–3 vs 4
Female, %	86.9	90	93.7	85.2	0.782	0.290
Age, yrs, mean ± SD	43.4 ± 15.3	44.8 ± 15.5	32.5 ± 10.6	42.7 ± 15.6	0.056	0.251
No. SLE ACR criteria met, mean ± SD	4.3 ± 0.8	4.8 ± 1.1	4.8 ± 1	4.7 ± 1.2	0.350	0.837
Disease duration, yrs, mean ± SD	8.9 ± 8.4	6.9 ± 7.9	6.1 ± 5.1	8.4 ± 9.1	0.612	0.371
Duration of neuropsychiatric symptoms, yrs, mean ± SD	2.1 ± 5.4	3.1 ± 5.2	1.5 ± 3.1	2.1 ± 3.1	0.629	0.142
No. (%) diagnosis NPSLE	12 (14.8)	19 (23.5)	12 (14.8)	38 (46.9)	0.068	**0.047**
Comparison of the number and frequencies of different CNS NPSLE ACR syndromes between clusters**						
Cerebrovascular disease	5 (17.2)	3 (10.3)	3 (10.3)	18 (62.1)	**0.03**	**0.009**
Headache	4 (16)	6 (28)	2 (8)	12 (48)	0.853	0.287
Psychosis	2 (11.8)	5 (29.4)	0 (0)	10 (58.8)	0.251	0.092
Seizure disorder	1 (7.7)	1 (7.7)	1 (7.7)	10 (76.9)	**0.048**	**0.007**
Cognitive dysfunction	8 (19.5)	8 (19.5)	6 (14.6)	19 (46.3)	0.338	0.262
Myelopathy	0 (0)	1 (14.3)	0 (0)	6 (85.7)	0.096	**0.019**
Mood disorder	4 (20)	7 (35)	2 (10)	7 (35)	0.904	0.365
Anxiety disorder	3 (12.5)	5 (20.8)	3 (12.5)	13 (54.23)	0.228	0.097
Acute confusional state	1 (14.3)	4 (57.1)	1 (14.3)	1 (14.3)	0.370	0.138
Movement disorder	0 (0)	1 (33.3)	0 (0)	2 (66.7)	0.71	0.367
Comparison of the number and frequencies of focal and diffuse CNS NPSLE ACR syndromes between clusters**						
Focal	7 (13.7)	10 (19.6)	6 (11.7)	28 (54.9)	0.06	**0.010**
Diffuse	9 (18.3)	12 (24.5)	6 (12.3)	22 (44.9)	0.341	0.341
Major focal syndromes†	5 (13.1)	5 (13.1)	4 (10.6)	24 (63.2)	**0.008**	**0.001**
Major diffuse syndromes††	5 (15.1)	11 (33.3)	3 (9.1)	14 (42.5)	0.920	0.539

* Chi-square test. P values in bold face are statistically significant. ** Values are the no. (%) of patients per syndrome. Demyelinating syndrome and aseptic meningitis did not occur. † Major focal syndromes include cerebrovascular disease, chorea, seizures, and myelopathy (according to ACR nomenclature). †† Major diffuse syndromes include acute confusional state, mood disorder, and psychosis (according to ACR nomenclature). LAC: lupus anticoagulant; ACR: American College of Rheumatology; CNS: central nervous system; NPSLE: neuropsychiatric systemic lupus erythematosus; SLE: systemic lupus erythematosus; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; aCL: anticardiolipin.

4. When clusters 1 to 3 (respectively, no specific antibodies, anti-dsDNA/anti-SSA/anti-SSB/anti-TRIM21, and anti-Sm/RNP) were grouped and compared to cluster 4 (anti-dsDNA/LAC/aCL IgM/IgG), additionally an association was found for myelopathy (p = 0.019) in cluster 4. No association between an individual autoantibody and an NPSLE manifestation was found, except for the following: aCL IgG with a NPSLE diagnosis in general (p = 0.019), headache (p = 0.004), or psychosis (p = 0.003) and LAC with a seizure disorder (p = 0.004). To our knowledge, this is the first report in NPSLE that involves cluster analyses on autoantibodies retrieved by multiplex testing. In our present study we found an association between a cluster of autoantibodies (anti-dsDNA/LAC/aCL IgG/IgM) and NPSLE. This association seems consistent with available literature[3,4,5]. This association was especially important in major focal syndromes and was stronger when patients with minor syndromes (headache, anxiety, cognitive dysfunction, and mild forms of depression) were excluded (p = 0.001).

On the other hand, our study failed to show any associations between the other autoantibodies analyzed with the microarray kit or clusters of these autoantibodies and NPSLE. The absence of more associations in our analyses hypothetically could also be due to the specific properties of this microarray kit, low numbers of patients per syndrome, or the fact that patients with NPSLE as a group represent several pathogenic processes.

Our data suggest that aPL are indispensable in the diagnostic investigations of NPSLE in daily practice. Further studies concerning these and other autoantibodies are required. Possibly, to study the role of (clusters of) autoantibodies more appropriately in NPSLE, their role in different pathogenic processes should be studied. Therefore our future work is aimed at finding associations between (clusters of) autoantibodies and advanced imaging results of the brain, as the best representative of tissue.

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Correction

Cluster Analysis of an Array of Autoantibodies in Neuropsychiatric Systemic Lupus Erythematosus

Zirkzee EJM, Magro Checa C, Steup-Beekman GM, Sohrabian A. Cluster analysis of an array of autoantibodies in neuropsychiatric systemic lupus erythematosus. J Rheumatol 2014;41:1720-1. The correct order of the authors' names should read: Zirkzee EJM, Magro Checa C, Sohrabian A, Steup-Beekman GM. We regret the error.

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