The effects of D-serine in synaptic plasticity have been attributed to its role as a gliotransmitter (Mothet et al., 2005; Panatier et al., 2006; Henneberger et al., 2010; Papouin et al., 2012), but the role of gliotransmission is still under debate (Agulhon et al., 2010, 2012; Nedergaard and Verkhratsky, 2012). Our study now suggests a role of neuronal D-serine released by Asc-1 in modulating LTP and synaptic NMDAR responses. Our data also indicate a role of neuronal glycine release in activating synaptic NMDARs. In this framework, Asc-1 provides a novel pathway for concurrent non-vesicular release of D-serine and glycine from neurons. Blockers of Asc-1 may therefore provide a new strategy to decrease NMDAR coactivation, whereas activators of the Asc-1 antiporter may be useful for conditions in which NMDAR function may be decreased, such as normal aging and schizophrenia.

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Original Paper

European Neurology

Eur Neurol 2014;71:42–48
DOI: 10.1159/000353982

Received: February 22, 2013
Accepted: June 23, 2013
Published online: November 27, 2013

Immunopathological Significance of Ovarian Teratoma in Patients with Anti-N-Methyl-D-Aspartate Receptor Encephalitis

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Key Words
Anti-N-methyl-D-aspartate receptor encephalitis · Ovarian teratoma · Immunopathological study · Limbic encephalitis · Paraneoplastic syndrome

Abstract
Background: The clinical importance of ovarian teratoma in anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis has been established, however investigations of ovarian teratoma in patients with anti-NMDAR encephalitis remain limited. ***Objective:*** To clarify differences in NMDAR distribution and lymphocyte infiltration in ovarian teratoma between patients with and without anti-NMDAR encephalitis. ***Methods:*** Participants initially comprised 26 patients with ovarian teratomas. NMDAR distribution and lymphocyte infiltration in ovarian teratomas were examined using immunopathological techniques. Clinical, laboratory, and radiological data were compared between patients showing the features of encephalitis. Anti-NMDAR antibodies in the serum and cerebrospinal fluid were also measured in encephalitis patients. ***Results:*** Neuronal tissues were obtained from ovarian teratomas in 22 patients (after excluding 4 patients who did not satisfy the inclusion criteria), and the presence of NMDA receptor subunits was revealed in all patients. Lymphocyte infiltration was more frequent in the encephalitis group (n = 3) than in the non-encephalitis group. In particular, dense B-lymphocyte infiltration near neural tissues was observed in the encephalitis group. ***Conclusions:*** Differences in lymphocyte infiltration in ovarian teratomas between anti-NMDAR encephalitis and non-encephalitis patients suggest the immunological importance of the ovarian teratoma as the site of antigen presentation in anti-NMDAR encephalitis.

In recent years, anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis has been receiving attention due to its clinical characteristics such as female predominance, limbic encephalitis-like clinical features, and the presence of an ovarian teratoma in over 50% of cases [1, 2]. Interestingly, this encephalitis has been associated with the antibody (Ab) against NMDAR subtypes 1 and 2B (NR1/NR2) heteromers of NMDAR in the serum and cerebrospinal fluid (CSF) [1, 3]. Several clinical features, such as responsiveness to immunotherapy and surgical teratoma removal and the presence of anti-NMDAR Abs in the serum and CSF, strongly suggest that autoimmunity may be central to the pathogenesis of anti-NMDAR encephalitis [4–6]. Although the relationship between anti-NMDAR encephalitis and ovarian teratomas has been considered, histological investigations of ovarian

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0014–3022/14/0712–0042539.50/0

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teratoma in anti-NMDAR encephalitis remain limited [5, 19].

We performed immunohistological analysis of ovarian teratomas from anti-NMDAR encephalitis patients and non-encephalitis controls to clarify differences in NMDAR distribution and lymphocyte infiltration.

Methods

Standard Protocol Approvals and Patient Consent

Prior to the initiation of this study, informed consent was obtained from each patient following a clear explanation of its purposes and methods. Ethics approval for this study was granted by the Saga University Ethics Committee.

Patients and Controls

Patients with ovarian teratomas hospitalized at Saga University Hospital between January 2004 and February 2010 were investigated in this study. All clinical data were obtained from Saga University Hospital medical records, and all formalin-fixed paraffin-embedded ovarian teratoma tissue blocks were obtained from the Department of Pathology, Saga University Faculty of Medicine. The clinical and laboratory parameters of patients with encephalitis in this study were as follows: age; symptoms; disease severity; laboratory data (thyroid functions, anti-thyroglobulin Abs, anti-nuclear Abs, anti-DNA Abs, Abs against several viruses including herpes simplex virus (HSV), CSF cell count and protein, immunoglobulin G in the CSF, and polymerase chain reaction testing for HSV DNA in CSF); radiological examinations, and electroencephalography. Exclusion criteria were as follows: an ovarian teratoma containing no neuronal tissue, and inappropriate tissue conditions for this study.

Detection of the Anti-NMDAR Antibody in the Serum and CSF

Methods for detecting the anti-NMDAR Ab have been described previously [8]. In brief, cDNA encoding NR1 and NR2B was ligated into expression vectors and transfected into human embryonic kidney (HEK)-293 cells in medium containing 10 mM MK-801 using Lipofectamine (Invitrogen, Carlsbad, Calif., USA). Twelve hours after transfection, HEK-293 cells were fixed in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (pH 7.4) for 20 min. After non-specific binding was blocked with 10% goat serum in phosphate-buffered saline, these cells were incubated with patient sera (1:40) or CSF (1:2) overnight at 4°C and then with fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin G (BD Biosciences, San Jose, Calif., USA) for 30 min at room temperature. SlowFade gold anti-fade reagent (Molecular Probes, Inc., Eugene, Oreg., USA) was applied to the slides and staining was observed under fluorescence microscopy.

Immunohistochemical Study of Ovarian Teratoma

Sections cut from formalin-fixed paraffin-embedded tissue blocks were used. The primary Abs used were NR1-C2 (dilution 1:100; Frontier Institute, Hokkaido, Japan), NR2B (dilution 1:100; Frontier Institute), SMI-31 (dilution 1:500; Convance, Emeryville, Calif., USA), ionized calcium-binding adaptor molecule 1 (IBA-1) (dilution 1:200; Abcam, Cambridge, Mass., USA), glial fibrillary acidic protein (GFAP) (dilution 1:100; Dako Cytomation, Glostrup, Denmark), Neurofilaments (dilution 1:100; Dako Cytomation), CD3 (prediluted; Nichirei Biosciences, Tokyo, Japan), CD4 (dilution 1:20; Nichirei Biosciences), CD8 (dilution 1:100; Dako Cytomation), and CD20 (dilution 1:100; Dako Cytomation). Slides were microwave-heated in ethylenediaminetetraacetic acid (pH 8) for antigen retrieval. The Envision+® System (Dako Cytomation) was used for the secondary Ab. Slides were visualized using diaminobenzidine tetrahydrochloride and nuclei were counterstained with hematoxylin. The Autostainer plus® automatic stainer (Dako Cytomation) was used to stain all Abs [2, 6]. The degree of staining for NMDA receptor Abs (NR1 and NR2B) was graded as follows: 0, no staining; focal (+), <30% cell staining; patchy (++), 31–60% cell staining, and diffuse (+++), >60% cell staining. To estimate the number of lymphocytes, a standard 3-point scoring system was used: low (–), intermediate (+), or high (++). The immunohistological results were independently scored by one pathologist and two neurologists.

Results

Twenty-six patients with ovarian teratomas were included in this study. These patients were divided into two groups: encephalitis group, 3 encephalitis patients with ovarian teratomas, and non-encephalitis group, 23 ovarian teratoma patients with no evidence of encephalitis. Four patients in the non-encephalitis group were excluded due to a lack of neural tissues in the ovarian teratoma (n = 2) or because of an insufficient sample state (n = 2).

Clinical Characteristics of Patients with Encephalitis

The mean age of the encephalitis group was 24.3 years (range 18–33) and that of the non-encephalitis group was 30.3 years (range 18–49). Clinical, laboratory, and radiological characteristics in the encephalitis group are shown in table 1.

Briefly, 2 of the 3 patients (cases 1 and 2) in the encephalitis group exhibited the typical clinical symptoms of anti-NMDAR encephalitis, including initial psychosis, subsequent central hypoventilation, intractable seizures, dysautonomia, and prominent orofacial dyskinesia. In contrast, 1 patient (case 3) exhibited psychosis mimicking limbic encephalitis, but never showed central hypoventilation, seizure, or orofacial dyskinesia. The presence of anti-neuronal Abs against NR1/NR2 heteromers of NMDAR was confirmed in both the serum and CSF; therefore, 3 patients in the encephalitis group were diagnosed with anti-NMDAR encephalitis. In contrast, ovarian teratoma patients in the non-encephalitis group showed no neurological or psychological symptoms according to their medical records.

Table 1. Clinical, laboratory, and radiological characteristics of patients with anti-NMDAR encephalitis

	Case 1 (severe group)	Case 2 (severe group)	Case 3 (mild group)
Age, years	24	34	18
Duration between disease onset to ovarian teratoma resection	70 days (hyperkinetic phase)	17 days (hyperkinetic phase)	20 days (psychotic phase)
Mechanical respiratory assistance	+	+	–
CSF findings			
Cells/ml	114	37	7
Protein, mg/dl	218	27	13
Anti-NMDAR antibody			
Serum	+	+	+
CSF	+	+	+
Abnormal head MRI findings	T2 hyperintensity (cerebrum)	T2 hyperintensity (cerebrum and cerebellum)	T2 hyperintensity (cerebellum)
Treatment			
Ovarian teratoma resection	+	+	+
Corticosteroids	+	+	+
Intravenous immunoglobulin	+	+	–
Plasma exchange	+	+	–

Histopathological and Immunohistochemical Findings in Ovarian Teratoma

Neural tissues were demonstrated in all teratoma samples from the encephalitis and non-encephalitis groups according to cell morphology and varying degrees of immunostaining for anti-SMI-31 Ab (a neuron-specific marker), anti-GFAP Ab (an astrocyte-specific marker), and anti-IBA-1 Ab (a microglia-specific marker) (fig. 1a–d, 2a–d). The presence of NMDAR in neuronal tissues was also revealed. In the encephalitis group, all neuronal tissues showed positive staining by anti-NR1 and anti-NR2 Ab with (++) intensity (fig. 1e, f). In contrast, the intensity of staining for anti-NR1 and anti-NR2 Ab-positive neural tissues in ovarian teratomas in the non-encephalitis group varied from (–) to (+++) (fig. 2e, f). Immunohistochemical data for neuronal tissue staining and the presence of NMDAR in the encephalitis and non-encephalitis groups are summarized in table 2.

Inflammatory cell infiltration around neural tissues was also observed in both the encephalitis group (n = 3) and non-encephalitis group (n = 2). Interestingly, inflammatory cell infiltration was observed in only 2 of 19 patients in the non-encephalitis group, whereas all 3 patients in the encephalitis group showed varying degrees of inflammatory cell infiltration. Both CD4-positive T lymphocytes and CD8-positive T lymphocytes were observed close to neural tissues (fig. 2g, h), and a predominance of CD4+ T-lymphocyte infiltration was observed in 4 of the 5 patients showing inflammatory cell infiltration (80%; all 3 patients in the encephalitis group and 1 of 2 patients in the non-encephalitis group) (fig. 2g, h). The presence of CD20-positive B lymphocytes was also observed around neural tissues in teratomas in the encephalitis and non-encephalitis groups. The state of B-lymphocyte infiltration was markedly denser in the encephalitis group than in the non-encephalitis group (fig. 1i, 2i). In addition, B-lymphocyte infiltration appeared to be adjacent to the site of NR1- and NR2B-positive neuronal tissues. This characteristic B-lymphocyte infiltration was observed among patients showing typical clinical features such as initial psychosis, subsequent central hypoventilation, intractable seizures, dysautonomia, and prominent orofacial dyskinesia. Immunohistochemical data for lymphocyte infiltration in the encephalitis and non-encephalitis groups are summarized in table 3.

Discussion

Immunotherapies such as intravenous methylprednisolone pulse therapy, intravenous immunoglobulin administration, and plasma exchange are well recognized as first-line treatments for anti-NMDAR encephalitis in the acute clinical phase. These therapeutic strategies strongly suggest that immunological pathogenesis lies in anti-NMDAR encephalitis. In addition, concerning anti-NMDAR en-

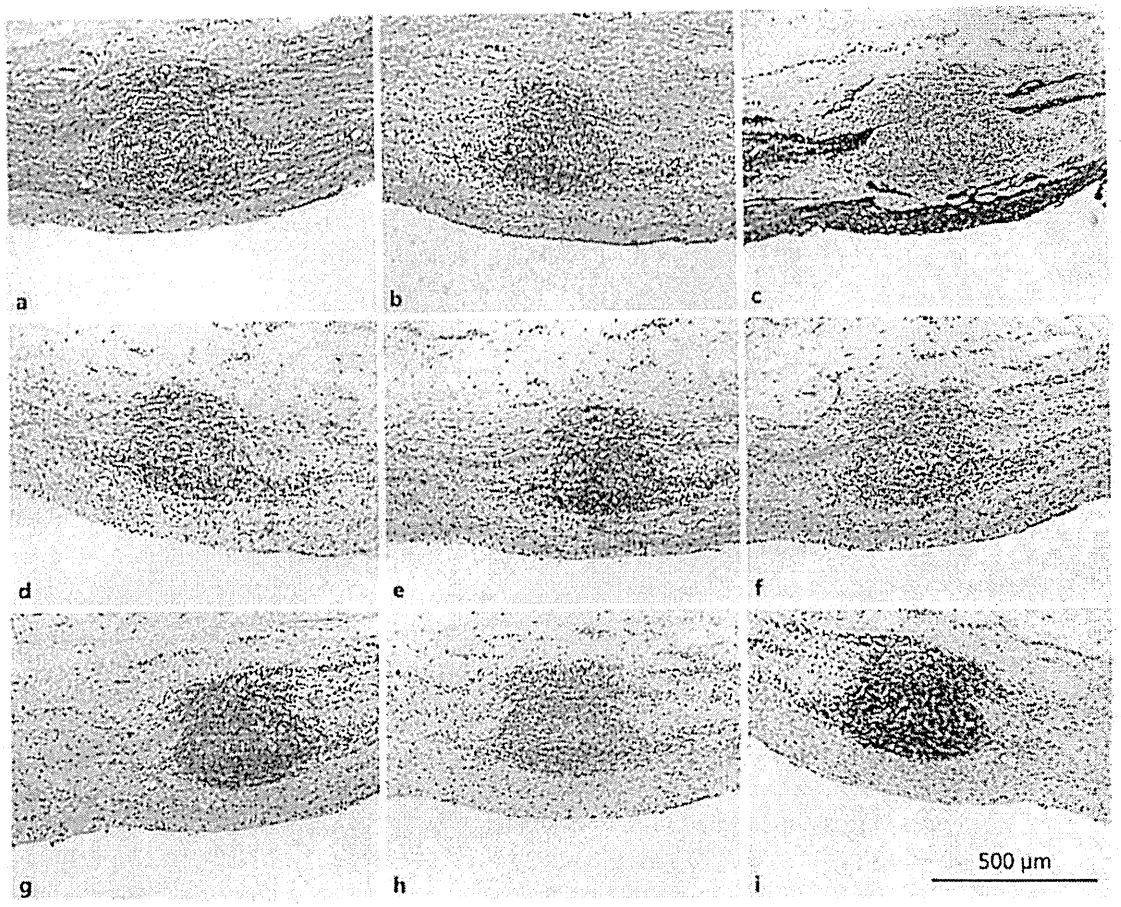

Fig. 1. Histopathological and immunohistochemical findings in ovarian teratomas with encephalitis (case 1). Neuronal tissues in the ovarian teratoma were demonstrated based on cell morphology (**a**). Immunostainings using anti-SMI-31 Ab (**b**), anti-GFAP Ab (**c**), and anti-IBA-1 Ab (**d**) confirmed the presence of several neuronal compartments in neuronal tissues. The presence of NMDAR in neuronal tissues was also demonstrated using anti-NR1 (**e**) and anti-NR2 Ab (**f**). Lymphocyte infiltration was revealed around neuronal tissues. CD4+ T lymphocytes (**g**) and CD8+ T lymphocytes (**h**) were observed around neuronal components. CD20-positive B lymphocytes were also observed around neuronal tissues with dense infiltration in the encephalitis group (**i**).

cephalitis complicated by ovarian teratoma, there have been reports that resection of the teratoma resulted in rapid and marked improvements in the condition, suggesting an involvement of ovarian teratoma in immune responses [7–12]. Ovarian teratoma appears to contribute to the pathogenesis of anti-NMDAR encephalitis [13–15]. Recent studies have shown that neuronal tissues in ovarian teratomas in anti-NMDAR encephalitis patients expressed NR2B and/or NR2A, and B- and T-lymphocyte infiltration has also been reported in the ovarian teratoma [2, 8]. In general, however, information on inflammatory cell infiltration in ovarian teratomas in patients with anti-NMDAR encephalitis is limited, and all except one report have described findings in the clinical recovery phase.

Similar to our results, Dabner et al. [19] reported pathological differences in ovarian teratomas between patients with anti-NMDAR encephalitis and non-encephalitis controls. Diffuse lymphoplasmacytic infiltrates were observed within the neurological matrix of ovarian teratomas in patients with anti-NMDAR encephalitis. Our study revealed the presence of neuronal tissues in ovarian teratomas in the encephalitis and non-encephalitis groups. NR1 and NR2B, as subunits of NMDAR, were also detected in neuronal tissues in both groups, which suggests that the presence of NMDAR itself is not a specific finding for anti-NMDAR encephalitis with ovarian teratoma. In addition, denser inflammatory cell infiltration around neural tissues in ovarian teratomas was observed in the encephalitis group.

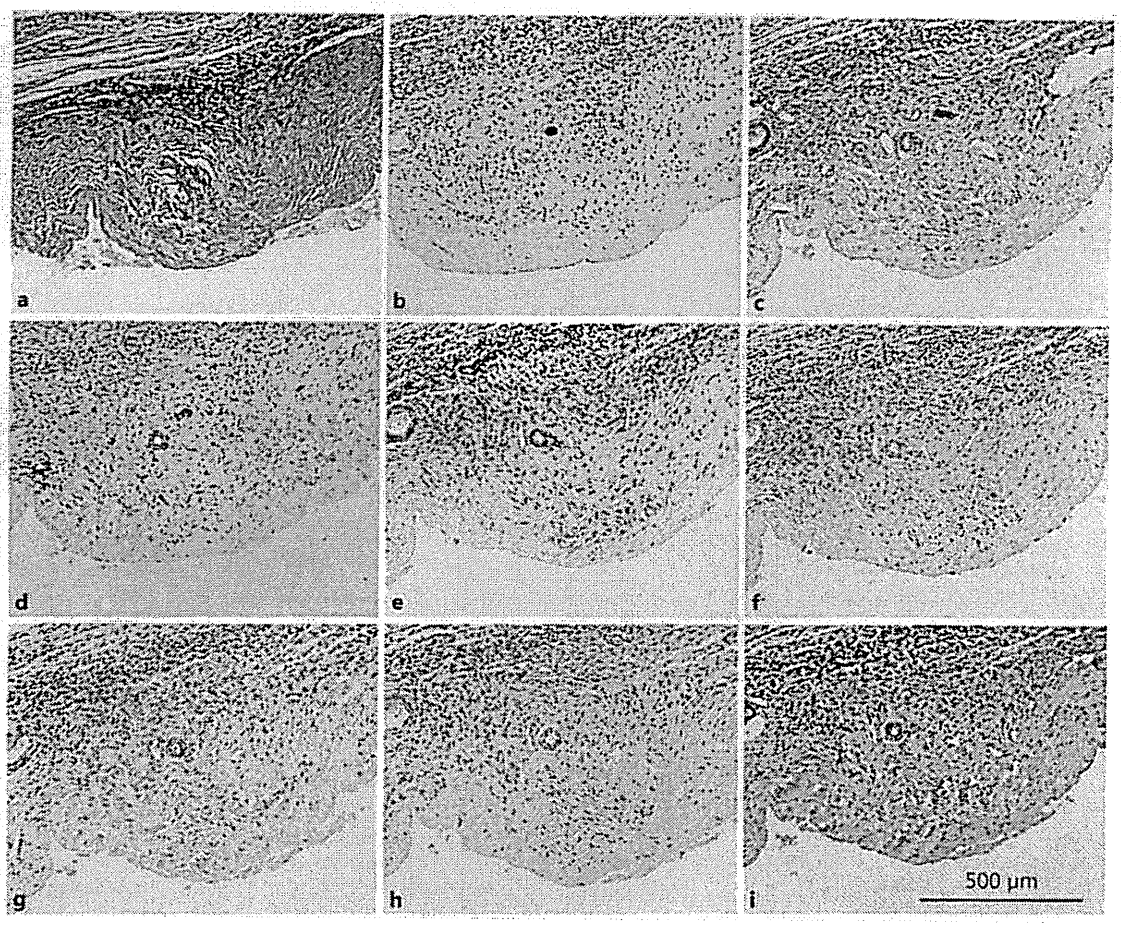

Fig. 2. Histopathological and immunohistochemical findings in ovarian teratomas with non-encephalitis. Neuronal tissues in the ovarian teratoma were demonstrated based on cell morphology (**a**). Immunostaining using anti-SMI-31 Ab (**b**), anti-GFAP Ab (**c**), and anti-IBA-1 Ab (**d**) confirmed the presence of several neuronal compartments in neuronal tissues. The presence of NMDAR in neuronal tissues was also demonstrated using anti-NR1 (**e**) and anti-NR2 Ab (**f**). Lymphocyte infiltration was revealed around neuronal tissues. CD4+ T lymphocytes (**g**) and CD8+ T lymphocytes (**h**) were observed around neuronal components. CD20-positive B lymphocytes were found around neuronal tissues with no or slight infiltration in the non-encephalitis group (**i**).

Furthermore, another study showed NR2B-related immunoreactivity in the cytoplasm of oocytes in normal ovaries. Taking these findings together, the presence of NMDAR itself is necessary, but not a sufficient condition in anti-NMDAR encephalitis. However, these results do not deny the importance of neuronal elements in an ovarian teratoma in the immunopathogenesis of anti-NMDAR encephalitis because differences in lymphocyte infiltration were also observed between ovarian teratomas in the encephalitis and non-encephalitis groups in this study.

In contrast with the ubiquitous presence of NMDAR in the ovary with or without encephalitis, the frequency and mode of lymphocyte infiltration differed markedly between the encephalitis and non-encephalitis groups. In particular, dense infiltration of CD20+ B lymphocytes around NR1- and NR2B-positive neuronal tissues represents a unique finding because this mode of infiltration was only observed in patients in the encephalitis group. This characteristic B-lymphocyte infiltration supports the immunopathogenesis of anti-NMDAR encephalitis against neuronal elements in ovarian teratomas, and differences in B-lymphocyte reactions against neuronal elements such as NMDAR could depend on some genetically predisposed individuals. Therefore, an ovarian teratoma may represent a site of antigen presentation for anti-NMDAR encephalitis patients with ovarian teratomas.

Table 2. Histopathological and immunohistochemical findings of ovarian teratoma in patients with anti-NMDAR encephalitis and non-encephalitis group

	Encephalitis group (n = 3)	Non-encephalitis group (n = 19)
Neuronal tissues staining, %		
SMI-31	100	100
GFAP	100	100
IBA-1	100	100
NMDAR staining, n (%)		
NR1 no stain (–)	0 (0)	1 (5)
Focal (+)	0 (0)	4 (18)
Patchy (++)	3 (100)	5 (25)
Diffuse (+++)	0 (0)	9 (45)
NR2B no stain (–)	0 (0)	5 (26)
Focal (+)	0 (0)	7 (42)
Patchy (++)	3 (100)	3 (16)
Diffuse (+++)	0 (0)	3 (16)
Lymphocyte infiltration, %	3 (100)	2 (10)

Table 3. Lymphocyte infiltration around neuronal tissues in the ovarian teratoma in patients with or without anti-NMDAR encephalitis

	T lymphocyte				B lymphocyte CD20
	CD3	CD4	CD8		
Encephalitis group					
Case 1	(+)	(+)	(+)	CD4 predominance	(++)
Case 2	(+)	(+)	(+)	CD4 predominance	(++)
Case 3	(+)	(+)	(+)	CD4 predominance	(+)
Non-encephalitis					
Group 1	(+)	(+)	(+)	CD4 = CD8	(+)
Group 2	(+)	(+)	(+)	CD4 predominance	(+)

(+) = Intermediate lymphocytes infiltration; (++) = highly lymphocytes infiltration.

Further investigations, such as differences in HLA constellations or microRNA expression profiles, are needed to clarify individual differences in genetic backgrounds.

On the other hand, systemic viral infection or mild inflammation affecting the ovary may create a trigger for NMDAR recognition, or the ovary itself may be important even in anti-NMDAR encephalitis patients without ovarian teratomas as a site of antigen exposure because NMDAR is present even in the ovary itself.

Although the number of patients in this study was small, the results, which showed differences in lymphocyte infiltration in ovarian teratomas between the anti-NMDAR encephalitis and non-encephalitis groups, suggest the importance and contribution of immunological mechanisms involving NMDAR to ovarian teratomas in anti-NMDAR encephalitis.

Acknowledgments

The authors are grateful to members of the Pathology Department in Saga University Faculty of Medicine for their helpful suggestions about ovarian pathology. Immunopathological analysis was contributed by Dr. Masatoshi Yokoyama and measurements of anti-NMDAR Abs in the serum and CSF were contributed by Dr. Yukitoshi Takahashi and Keiko Tanaka. This study was supported in part by grants from the Ministry of Health, Labour and Welfare, Japan.

Disclosure Statement

The authors have no conflicts of interest to disclose.

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A Novel Treatment-Responsive Encephalitis with Frequent Opsoclonus and Teratoma

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Among 249 patients with teratoma-associated encephalitis, 211 had N-methyl-D-aspartate receptor antibodies and 38 were negative for these antibodies. Whereas antibody-positive patients rarely developed prominent brainstem–cerebellar symptoms, 22 (58%) antibody-negative patients developed a brainstem–cerebellar syndrome, which in 45% occurred with opsoclonus. The median age of these patients was 28.5 years (range = 12–41), 91% were women, and 74% had full recovery after therapy and tumor resection. These findings uncover a novel phenotype of paraneoplastic opsoclonus that until recently was likely considered idiopathic or postinfectious. The triad of young age (teenager to young adult), systemic teratoma, and high response to treatment characterize this novel brainstem–cerebellar syndrome.

ANN NEUROL 2014;75:435–441

The discovery of anti–N-methyl-D-aspartate receptor (NMDAR) encephalitis in 2007[1] has brought attention to a relationship between systemic teratomas and autoimmune encephalitis. Since 2007, we have studied 249 patients with teratoma-associated encephalitis; most of these patients had antibodies against the NR1 subunit of the NMDAR, but 38 were NMDAR antibody negative. When these 38 patients were compared with those with NMDAR antibodies, a novel brainstem–cerebellar syndrome that frequently associates with opsoclonus emerged. The current study describes the clinical differences between NMDAR antibody-positive and antibody-negative patients with systemic teratoma, and focuses on the novel brainstem–cerebellar syndrome and the subgroup of patients with opsoclonus.

Patients and Methods

From January 2007 until September 2012, serum and CSF of 249 patients with teratoma-associated encephalitis were studied at the Department of Neurology, Hospital of the University of Pennsylvania and at the Neurology Service, Hospital Clinic, August Pi i Sunyer Biomedical Research Institute, University of Barcelona. The presence of a systemic teratoma was confirmed pathologically in 234 patients and radiologically in 15. Information was obtained by the authors or provided by referring physicians at symptom onset and at regular intervals during the course of the disease using a comprehensive questionnaire that includes all symptoms shown in the Figure.[2] Sera and cerebrospinal fluid (CSF) were examined for antibodies to NMDA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, γ-aminobutyric acid (B), and mGluR5 receptors, LGI1, Caspr2, onconeuronal proteins (Hu, CRMP5, Ma1–2, amphiphysin), and GAD65, using reported techniques including brain immunohistochemistry, immunoblot, and cell-based assays.[3–5] Patients without NMDAR antibodies were further studied for antibodies to dipeptidyl-

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Additional Supporting Information may be found in the online version of this article.

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.23917

Received Feb 7, 2013, and in revised form Mar 28, 2013. Accepted for publication Apr 19, 2013.

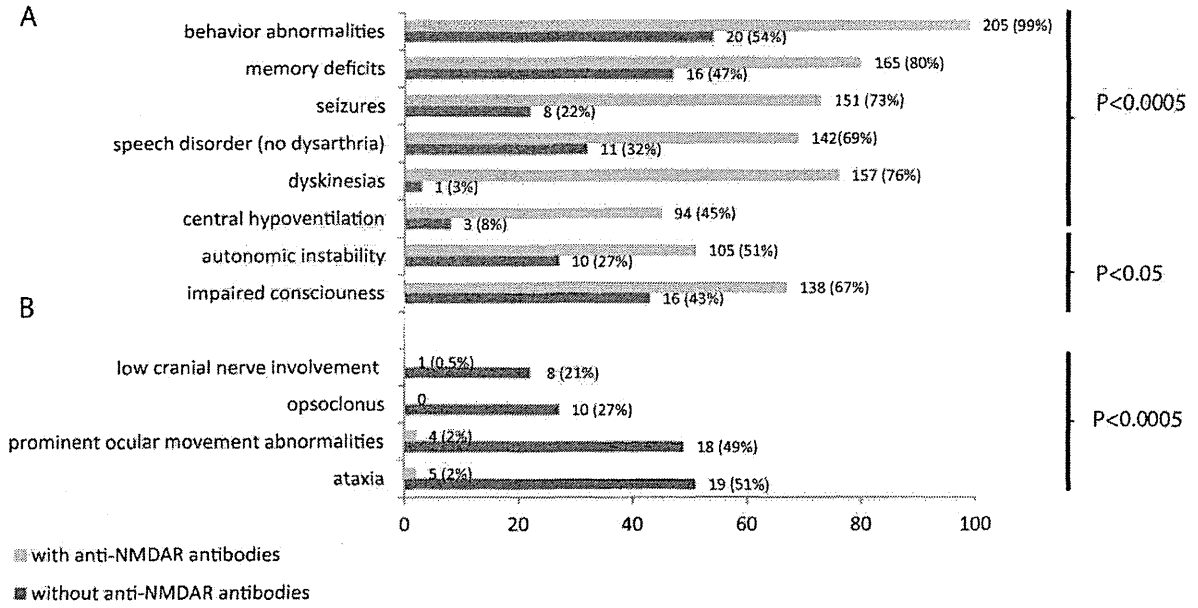

FIGURE 1: Comparison of symptoms of patients with teratoma-associated encephalitis and N-methyl-D-aspartate receptor (NMDAR) antibodies with those without NMDAR antibodies. (A) Patients without NMDAR antibodies (indicated in dark gray) less frequently developed symptoms considered characteristic of anti-NMDAR encephalitis (behavioral abnormalities, memory deficits, seizures, dyskinesias, speech disorder, and central hypoventilation, all $p<0.0005$, and impaired level of consciousness and autonomic dysfunction, $p<0.05$). (B) In addition, patients without NMDAR antibodies more frequently developed brainstem–cerebellar symptoms and opsoclonus, which are rare in anti-NMDAR encephalitis (all $p<0.0005$). From 1 patient without NMDAR antibodies and 4 with antibodies, detailed clinical information was not available, and these patients were excluded from analysis; in 3 additional patients without NMDAR antibodies, information for memory deficits and speech disorder was not available.

peptidase-like protein-6 (DPPX), α1-glycine receptor, D2 subunit of the dopamine receptor, and unknown cell-surface antigens using reported techniques.[3–5]

Outcome was assessed with the modified Rankin scale (mRS),[6] grading it as full recovery (mRS = 0), substantial improvement (mRS = 1–2), partial improvement (mRS > 2 after having had at least 1 point of improvement), and no improvement. Three patients without NMDAR antibodies have been previously reported.[7–9] Studies were approved by the internal review boards of the University of Pennsylvania and University of Barcelona.

Statistical Analysis

Comparative analyses between patients with and without NMDAR antibodies were performed with SPSS version 20 (IBM, Armonk, NY), using the Fisher exact test for contingency tables and Mann–Whitney *U* tests for continuous variables.

Results

Two hundred eleven patients were found to have NMDAR antibodies, and 38 were negative for these antibodies. Compared with antibody-positive patients, the 38 patients without NMDAR antibodies showed no differences with respect to gender and age of symptom onset (NMDAR antibody-negative patients: 92% female, median age = 28 years [interquartile range (IQR) = 20–32, range = 12–55] vs antibody-positive patients: 99% female, median age = 25 years [IQR = 19–30, range = 7–65], $p = 0.05$ and $p = 0.11$, respectively). However, significant differences were identified with respect to symptom presentation and repertoire of symptoms during the first month of the disease (see Fig 1). Whereas 18 (47%) patients without NMDAR antibodies initially presented with brainstem–cerebellar dysfunction, this presentation did not occur in any of the patients with NMDAR antibodies ($p < 0.0005$). In contrast, whereas 144 of 211 (68%) patients with NMDAR antibodies presented with psychosis and behavioral abnormalities, this presentation occurred only in 4 of 38 (11%) patients without these antibodies ($p < 0.0005$).

The Figure shows that during the first month of the disease, 76% of the patients with NMDAR antibodies developed dyskinesias, often involving the face and mouth, whereas only 1 (3%) patient without these antibodies developed dyskinesias, without affecting the face and mouth ($p < 0.0005$); similar differences were seen for most symptoms typical of anti-NMDAR encephalitis. In contrast, 22 of 38 (58%) patients without NMDAR

TABLE 1. Clinical Features in Patients with Brainstem–Cerebellar Syndrome and Systemic Teratoma without N-Methyl-D-Aspartate Receptor Antibodies

Opsoclonus	Patient No.	Age, yr/Sex	Main Symptoms	Neurologic Symptoms before Tumor Diagnosis	Brain MRI	CSF	Treatment	Response to Treatment	Immunological Studies with Cultures of Neurons
With	1	20/F	Opsoclonus–myoclonus, limb ataxia, dysarthria, meningeal signs, drowsiness, tonic seizures, autonomic instability (ileus, urinary retention)	Yes	Meningeal enhancement	182 WBC/μl (87% L), 99mg/dl protein; repeat study: 326 WBC/μl, 159mg/dl protein	Tumor removal, steroids	Complete, related to immunotherapy	Negative
	2	15/F	Opsoclonus–myoclonus, ataxia, drowsiness, vomiting, blurred vision	Yes	Normal	37 WBC/μl, 64mg/dl protein	Tumor removal, steroids, IVIg	Complete, related to tumor removal	Negative
	3	26/F	Opsoclonus–myoclonus, ataxia, dysarthria, aphasia, 3 days after tumor removal	No	Normal	72 WBC/μl (69% L), 49mg/dl protein, OB positive	Steroids, IVIg, plasma exchange (3×), rituximab (3 cycles); 1 cycle of bleomycin, etoposide, and carboplatin; 3 cycles of etoposide and cisplatin	Partial response to steroids, IVIg, plasma exchange; complete recovery after chemotherapy and rituximab	Negative
	4	31/F	Opsoclonus–myoclonus, ataxia, tinnitus	Yes	Normal	Mild pleocytosis with increased lymphocytes and protein concentration	Tumor removal (bilateral), steroids, IVIg, plasma exchange, chlorambucil	Complete, related to immunotherapy and tumor removal; relapsed 7 years later with mild ataxia and memory deficits	Reactivity of serum with cell surface of neurons
	5	22/F	Opsoclonus–myoclonus, ataxia, abnormal behavior, impaired consciousness; severe bradycardia requiring sinus pacemaker	Yes	Normal	10 WBC/μl, 57mg/dl protein, OB positive	Tumor removal, steroids	Partial, related to immunotherapy; complete after tumor removal	Negative
	6	24/F	Opsoclonus without myoclonus, truncal ataxia, vertigo, abdominal pain, generalized weakness, hyporeflexia	Yes	Normal[a]	<5 WBC/μl, <45mg/dl protein	Tumor removal, steroids, IVIg	Complete, related to immunotherapy	Negative
	7	30/F	Opsoclonus without myoclonus, dizziness, meningeal signs, seizures, abnormal behavior (not psychotic), weakness, hyporeflexia, central hypoventilation	Yes	1st normal; repeat study: brainstem edema and meningeal enhancement	134 WBC/μl (88% L), 88 mg/dl protein; repeat study: 414 WBC/μl (97% L), 110mg/dl protein	Steroids, IVIg, plasma exchange	Complete, related to immunotherapy; remained with motor weakness 3 months after disease onset	Negative
	8	29/F	Opsoclonus–myoclonus, sense of unsteadiness and body "shakiness" (26th week of pregnancy)	No	Not done	<5 WBC/μl, <45mg/dl protein, OB positive	Tumor removal, steroids	Complete, related to immunotherapy	Negative

TABLE 1. (Continued)

Opsoclonus	Patient No.	Age, yr/Sex	Main Symptoms	Neurologic Symptoms before Tumor Diagnosis	Brain MRI	CSF	Treatment	Response to Treatment	Immunological Studies with Cultures of Neurons
	9	28/F	Opsoclonus–myoclonus, dysarthria, ataxia, behavioral disinhibition, hypersexuality, hyperphagia, cognitive decline	No	Normal	12 WBC/μl, <45mg/dl protein	Tumor removal, steroids, IVIg, plasma exchange, azathioprine	Partial, related to immunotherapy; improved dysarthria and opsoclonus; ataxia still improving at last follow-up (15 months)	Reactivity of serum with cell surface of neurons
	10	32/F	Opsoclonus–myoclonus, dysarthria, diplopia, ataxia	Yes	Normal	30 WBC/μl, <45mg/dl protein	Tumor removal, steroids, IVIg, rituximab (4 doses)	Partial, related to immunotherapy and tumor removal; mild ataxia and dysarthria at last follow-up (13 months)	Negative
Without	11	19/F	Right hand tremor, ataxia, bilateral dysdiadochokinesia, dysmetria	Yes	Normal	124 WBC/μl, <45mg/dl protein	Tumor removal, steroids	Complete, related to immunotherapy and tumor removal	Negative
	12	32/F	Subacute tremor, unsteady gait	Yes	Normal	<5 WBC/μl, <45mg/dl protein	Tumor removal	Complete, related to tumor removal	Negative
	13	31/F	Subacute onset of vomiting, nystagmus, ataxic gait, dysarthria, myoclonus; all symptoms resolved after removal of ovarian teratoma, but the patient developed abnormal behavior, memory deficit, labile affect, and optic neuritis; recurrence of symptoms and oculomotor paresis 4 months later	Yes	1st normal; at clinical relapse 7 months later: abnormality at the level of oculomotor nuclei	16 WBC/μl, <45mg/dl protein, OB negative	Tumor removal, steroids, IVIg	Partial with tumor removal, complete with immunotherapy; relapse 8 months later (4 months after recovery)	Negative
	14	23/M	Cerebellar ataxia	Yes	NA	NA	NA	NA	Negative
	15	33/F	Severe cerebellar ataxia	Yes	NA	NA	Tumor removal	NA	Negative
	16	15/F	Left side ataxia, dysarthria, paresthesias, dysdiadochokinesia with left hand (onset 1.5 months after tumor removal)	No	Normal brain MRI and PET scan	<5 WBC/μl, <45mg/dl protein	Not treated	Symptoms stable with no improvement 4 months after presentation	Reactivity of CSF with cell surface of neurons

TABLE 1. (Continued)

Opsoclonus	Patient No.	Age, yr/Sex	Main Symptoms	Neurologic Symptoms before Tumor Diagnosis	Brain MRI	CSF	Treatment	Response to Treatment	Immunological Studies with Cultures of Neurons
	17	33/F	6-week episode of severe cerebellar ataxia that resolved without any specific treatment; relapse 2 years later: ataxia and memory problems; ovarian teratoma found	Yes	Normal	<5 WBC/μl, <45mg/dl protein	Not treated	Complete without treatment, but relapsed 2 years later	Negative
	18	36/F	Subacute ataxia, memory problems, confabulation, bilateral intention tremor, bilateral gaze directed nystagmus	Yes	Diffuse bilateral atrophy, enlarged ventricles (history of alcohol abuse)	<5 WBC/μl, <45mg/dl protein, OB negative	NA	NA	Negative
	19	28/F	Suspected viral meningoencephalitis (drowsiness, fever, headache), followed by seizures, brainstem symptoms, ataxia, cognitive and behavioral abnormalities	Yes	1st normal; 1 year later: diffuse brain atrophy (predominant in cerebellum)	66 WBC/μl, 126mg/dl protein	Tumor removal	No response; dependent for activities of daily living (dressing, feeding, ambulation) due to cognitive deficits and tetraparesis	Negative
	20	12/M	Left side ataxia, bilateral tremor, weakness, short-term memory loss; status epilepticus after testis teratoma removal	Yes	FLAIR hyperintensities in limbic region; right cortical atrophy	13 WBC/μl, <45mg/dl protein, OB positive	Tumor removal, steroids, IVIg	Partial, ataxia and coordination problems 4 months after onset	Negative
	21	41/F	Subacute diplopia, ophthalmoplegia, impaired consciousness; left ovarian teratoma discovered at workup of encephalitis (history of a right ovarian teratoma removed 10 years earlier)	Yes	Normal	25 WBC/μl, 85mg/dl protein	IVIg, plasma exchange	Complete, related to immunotherapy	Reactivity of serum with cell surface of neurons
	22	31/F	Myoclonus of lips, diplopia, confusion, catatonia, orthostatic hypotension	Yes	Increased FLAIR signal in medial temporal lobes	106 WBC/μl (98% L), 63.2mg/dl protein	Tumor removal, steroids	Complete, related to tumor removal and immunotherapy	Negative

[a]Normal brain MRI, but decreased degree of tracer accumulation in the brainstem and bilateral cerebral hemispheres on single photon emission computed tomography.
CSF = cerebrospinal fluid; F = female; FLAIR = fluid-attenuated inversion recovery; IVIg = intravenous immunoglobulin; L = lymphocyte; M = male; MRI = magnetic resonance imaging; NA = not available; OB = oligoclonal bands; PET = positron emission tomography; WBC = white blood cell count.

antibodies developed brainstem–cerebellar symptoms during the first month of the disease, 10 (45%) of them with opsoclonus, whereas these symptoms rarely occurred in patients with NMDAR antibodies. The identification of a predominant brainstem–cerebellar syndrome led us to focus on this disorder and the subgroup of patients with opsoclonus, both described below (the other 16 patients are shown in the Supplementary Table).

Brainstem–Cerebellar Syndrome

The median age of the 22 patients with brainstem–cerebellar symptoms was 28.5 years (IQR = 22–32, range = 12–41). Twenty (91%) were female, all with ovarian teratoma; 2 male patients had testicular teratoma. Main symptoms included ataxia in 86%, opsoclonus–myoclonus in 45% (described below), dysarthria in 36%, decreased level of consciousness in 32%, diplopia or ophthalmoparesis in 18%, and seizures in 18%. Other symptoms are listed in the Table 1.

Neurological symptoms developed before tumor diagnosis in 18 patients (82%; median = 1 month, IQR = 0.9–2 months, range = 3 days to 24 months) and after tumor diagnosis in 4 (10 days and 1.5, 2, and 3.5 months, respectively). Two of these 4 patients had the tumor removed 3 days and 1.5 months before developing encephalitis, respectively. All patients had mature teratomas, except 1 who had an immature ovarian teratoma. Serum of 3 patients (2 with opsoclonus) and CSF of another patient showed weak immunolabeling of cultures of rat neurons (data not shown); no antibodies were identified in the other patients.

Treatment and follow-up information was available for 19 (86%) patients, including all patients with opsoclonus (described below). Fifteen (79%) received immunotherapy, 13 of them with tumor resection; 2 had tumor resection without immunotherapy, and 2 were not treated (1 had tumor removal before developing encephalitis). With a median follow-up of 15 months (range = 3–84), 14 patients (74%) had full recovery, 3 (16%) had partial improvement, and 2 had no improvement (1 of them was not treated). Two patients with complete recovery and 1 with partial recovery relapsed 2 years, 7 years, and 8 months after disease onset, respectively.

Opsoclonus–Myoclonus Syndrome

Ten women (median age = 27 years, IQR = 22–30, range = 15–32) with brainstem–cerebellar syndrome developed opsoclonus; accompanying symptoms are listed in the Table 1. Four had prodromal fever or viral-like symptoms, and another one was 26 weeks pregnant. Symptoms developed before the tumor diagnosis in 7 (median = 1 month, IQR = 0.1–1.5 months, range = 3 days to 2 months) and after tumor diagnosis in 3 (10 days, 2 months, and 3.5 months, respectively). One of these 3 patients had undergone tumor resection 3 days before developing opsoclonus; the other 2 patients had not had tumor treatment.

At symptom onset, 7 patients had CSF lymphocytic pleocytosis (median = 37 white blood cells/μl, range = 10–182), 6 had increased protein concentration (median = 64/dl, range 49–100), and 3 of 3 had oligoclonal bands. Brain magnetic resonance imaging and electroencephalographic studies were abnormal in 2 of 9 and 3 of 5 patients (see Table 1).

All patients were treated with methylprednisolone: 3 alone, 3 combined with intravenous immunoglobulin (IVIg), and 4 with IVIg and plasma exchange. Two patients received rituximab after failing initial immunotherapy, and 1 received azathioprine (see Table 1). Nine patients had resection of the teratoma; pathological studies showed mature teratoma in 8, including 1 with bilateral teratomas, and immature teratoma in 1. Chemotherapy was used in 2 patients (see Table 1). Valproic acid, clonazepam, levetiracetam, or phenobarbital did not control the opsoclonus–myoclonus (data not shown).

The median time of follow-up was 19.5 months (IQR = 6–39, range = 3–84). Eight patients had full recovery, and 2 had mild residual dysarthria and ataxia at 13- and 15-month follow-up, respectively. Six of the 8 patients with full recovery became asymptomatic within the first 3 months of treatment, and the other 2 patients within 6 and 12 months, respectively.

Discussion

This study shows that patients with systemic teratoma can develop several forms of encephalitis without NMDAR antibodies, among which a syndrome that associates with brainstem–cerebellar symptoms stands out. Almost 50% of patients with this syndrome developed opsoclonus in association with the triad of young age (teenager to young adult), presence of an ovarian teratoma, and high response to treatment. The subacute presentation of symptoms, frequent CSF pleocytosis, and response to immunotherapy coupled with the detection of antibodies to neuronal cell-surface antigens in some patients suggest an immune-mediated pathogenesis.

All patients with opsoclonus were young women (aged 15–32 years), considered too young for carcinoma-associated opsoclonus, which usually occurs in patients >50 years old,[10] and too old for neuroblastoma-associated opsoclonus, which usually affects children <5 years old.[11] It is likely that this type of opsoclonus has been previously considered idiopathic or postinfectious and

that the presence of a teratoma was missed or not felt to be related.

Compared with patients with anti-NMDAR encephalitis, those without these antibodies were less likely to initially present with psychosis and behavioral change. Although there was overlap of some symptoms, such as limbic dysfunction and psychiatric manifestations, the frequency of other symptoms, such as dyskinesias, rarely occurred in patients without NMDAR antibodies. In contrast, patients with anti-NMDAR encephalitis did not initially present with brainstem–cerebellar dysfunction or opsoclonus. Of note, ataxia can be a presentation of anti-NMDAR encephalitis in children[2,12]; this is not reflected here, because young children usually do not have teratomas.

This study has several practical implications. Any teenager or young adult, especially if female, who develops subacute brainstem–cerebellar symptoms or opsoclonus–myoclonus suspected to be immune-mediated (because of the rapid onset of symptoms and/or CSF pleocytosis) should be investigated for a teratoma in the ovary (or testes for male patients). Detection of a teratoma should prompt its removal along with the use of immunotherapy (most patients described here received steroids, IVIg, and/or plasma exchange). A limitation of this study is that it is retrospective; future studies will establish the frequency of these disorders and may identify patients with higher levels of cell-surface antibodies that could lead to the characterization of the antigens.

Acknowledgment

This work was supported in part by grants from the NIH (RO1NS077851 [NINDS], J.D.; RO1MH094741 [NIMH], J.D.); National Cancer Institute (RO1CA89054, J.D.); Fundació la Marató de TV3 (J.D.); and Fondo de Investigaciones Sanitarias, Madrid, Spain (PI11/01780, J.D.; PI12/00611, F.G.); a fellowship from the Dutch Cancer Society (KWF2009–4451, M.J.T.); and a McKnight Neuroscience of Brain Disorders award (J.D.). T.A. received a personal grant from the Instituto Carlos III (FI12/00366).

We thank Dr I. Kawachi, Dr N. Singhal, Dr F. Cendes, Dr J. Gállego Pérez-Larraya, Dr Y. Hayashi, Dr E. Deegan, J. Honnorat, and V. Rogemond for providing clinical information; other physicians and family members of patients who provided clinical information; Dr M. R. Rosenfeld for critical review of the manuscript; and E. Aguilar for anti-NMDAR antibody testing.

Potential Conflicts of Interest

J.D.: grants/grants pending, Euroimmun, NIH; patents, royalties, Athena Diagnostics Euroimmun; editorial board, *Up-To-Date*.

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Clinical Neurology and Neurosurgery 118 (2014) 44–48

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Brain perfusion SPECT in limbic encephalitis associated with autoantibody against the glutamate receptor epsilon 2

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ARTICLE INFO

Article history:
Received 5 August 2012
Accepted 14 December 2013
Available online 27 December 2013

Keywords:
Non-herpetic limbic encephalitis
Autoantibody against the glutamate receptor epsilon 2
Brain perfusion SPECT
eZIS analysis

ABSTRACT

Objectives: The aim of this study was to elucidate the single-photon emission computed tomography (SPECT) pattern at the acute stage of disease in non-herpetic limbic encephalitis (NHLE) patients associated with the N-methyl-D-aspartate-type glutamate receptor epsilon 2 (GluR ϵ2) autoantibody using *Z*-score imaging system (eZIS) analyses.
Methods: Brain magnetic resonance imaging (MRI) and brain perfusion SPECT using technetium-99 ethyl cysteinate dimer (^{99m}Tc-ECD) were performed in eight patients with NHLE (5 men and 3 women; mean age 48.8 ± 22 years) within 20 days after clinical onset.
Results: All patients had various clinical limbic-associated symptoms and no evidence of herpes simplex infection or systemic malignancies. Two of eight patients showed abnormally hyperintense lesions on diffusion-weighted images and significant hyperperfusion in ipsilateral cerebral cortex on eZIS analysis, whereas other patients showed normal MRI findings and significant hypoperfusion in one or both sides of the limbic and paralimbic areas.
Conclusion: We suggest that ^{99m}Tc-ECD SPECT study using eZIS analyses may be helpful to detect the neuronal dysfunction, particularly in NHLE patients without abnormal MRI findings.

1. Introduction

Limbic encephalitis is clinically characterized by limbic-associated symptoms, such as confusion, short-term memory loss, psychiatric symptoms, and seizures, in addition to acute encephalitis symptoms, and includes a paraneoplastic and non-herpetic limbic encephalitis (NHLE) [1–7]. NHLE was recently identified as a new type of limbic encephalitis in Japan, and is characterized by the lack of evidence of herpes simplex virus (HSV) infection and paraneoplastic disease process [4,5]. Some patients with NHLE show positive autoantibody against the N-methyl-D-aspartate-type glutamate receptor epsilon 2 (GluR ϵ2) in cerebrospinal fluid [6,7]. GluR ϵ2 channels have been implicated in synaptic plasticity and localization associated with neural development and learning [8]. Although it remains unclear whether the autoantibody against GluR ϵ2 is the cause or the result of NHLE, the presence of this antibody suggests the autoimmune pathogenic mechanism [6,7]. Accurate and early diagnosis of NHLE is important for deciding on treatment and management. Brain magnetic resonance imaging (MRI) is highly sensitive for detecting early changes in NHLE and shows abnormal findings in one or both medial temporal lobes [6,7,9]. Some patients, however, have normal MRI findings and it is difficult to diagnose these patients based solely on clinical examination. Therefore, other sensitive methods to detect the neuronal dysfunction in limbic system are needed. Brain perfusion single-photon emission computed tomography (SPECT) provides an indirect marker of neuronal function. SPECT studies in HSV encephalitis reported the abnormal uptake of technetium-99 ethyl cysteinate dimer (^{99m}Tc-ECD) or technetium-99m hexamethylpropylamine-oxime (^{99m}Tc-HMPAO) in affected regions [10,11]. To our knowledge, few SPECT imaging studies have been performed in NHLE patients associated with GluR ϵ2 autoantibody.

Here we evaluated cerebral blood flow during the acute stage in NHLE patients associated with GluR ϵ2 autoantibody using *Z*-score imaging system (eZIS). The eZIS programs are statistical analysis techniques for diagnosis of brain perfusion SPECT images that can be used to objectively evaluate rCBF [12]. The aim of this study was to elucidate SPECT pattern in NHLE patients associated with GluR ϵ2 autoantibody.

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http://dx.doi.org/10.1016/j.clineuro.2013.12.006

Table 1
Summary of the clinical and demographic characteristics.

Patient	Age/sex	Psychiatric symptoms	Convulsive state	CSF		SPECT study[a]
				Cells (mm^3)	Protein (mg/dl)	
1	30/M	Memory loss and agitation	+	9	57	15
2	58/M	Hallucination and delirium	+	1	20	13
3	36/F	Disorientation, delirium, agitation, and generalized seizures	–	55	45	10
4	80/M	Delirium, agitation, and generalized seizures	–	1	35	9
5	57/M	Disorientation and agitation	–	26	84	2
6	76/F	Memory loss, disorientation, and delirium	–	4	134	13
7	24/M	Agitation and generalized seizures	–	30	27	4
8	29/F	Disorientation, anxiety, delirium, and generalized seizures	–	1	31	20

M, male; F, female; CSF, cerebrospinal fluid.
[a] Duration from clinical onset to study (days).

2. Methods

2.1. Subjects

Eight patients with NHLE (5 men and 3 women; mean age 48.8±22 years) were admitted to the Department of Internal Medicine III, Oita University, and both brain MRI and brain perfusion SPECT were performed between 2007 and 2010. Autoantibody against GluR ϵ2 in cerebrospinal fluid (CSF) was detected in all patients using enzyme-linked immunosorbent assay (ELISA). The diagnosis criteria of NHLE was based on the previous reports as follows [4,5]: (1) encephalitis symptoms, such as fever and disturbed consciousness, (2) limbic-associated symptoms, such as short-term memory loss, seizure, and various psychiatric symptoms, (3) negative HSV DNA in CSF by polymerase chain reaction and negative antibodies in the CSF for HSV, varicella-zoster virus, Epstein-Barr virus, and human herpes virus-6 determined by the ELISA, (3) lesions of the temporal lobe on MRI, (4) absence of systemic malignancy and collagen disease, (5) no bacteria or fungi in CSF culture, and (6) the exclusion of all other neurologic, vascular, metabolic, toxic, and drug-induced disorders. Moreover, we included patients without abnormal MRI findings, regardless of the presence of limbic-associated symptoms. All patients showed no malignancy on contrast enhanced computed tomography (CT) of the chest, abdomen, and pelvis. Information regarding age, sex, neurologic symptoms, laboratory tests, including the complete blood count, urine components, blood biochemistry, thyroid function, and tumor markers, and CSF analysis and electroencephalography findings were extracted from the medical records. Brain MRI and brain perfusion SPECT were performed in the interictal state. The electroencephalogram was not performed at the time of SPECT study.

2.2. Brain MRI

MRI was performed with a 1.5 T scanner (Excelart Vantage; Toshiba Medical System Corp., Tokyo, Japan), using spin echo sequences. T1-weighted image, T2-weighted image, and diffusion-weighted image were obtained on axial slices. The pulse sequence being repletion time (TR) 500–550 ms/echo time (TE) 12 ms for T1-weighted image, TR 4000–5000 ms/TE 90 ms for T2-weighted image, and TR 5000–6000 ms/TE 100 ms for diffusion-weighted image. The section thickness was 5 mm with an intersection space of 1.2 mm.

2.3. Brain SPECT imaging

Patients were asked to assume a comfortable supine position with eyes closed in quiet surroundings. After intravenous injection of ^{99m}Tc-ECD (600 MBq, FUJIFILM RI Pharma Co., Tokyo, Japan), its passage from the heart to the brain was monitored using a rectangular large field gamma camera (E. Cam Signature, Toshiba Medical, Tochigi, Japan). Data comprising a sequence of 120 frames was acquired at a rate of one frame per second with a 128 × 128 matrix. Ten minutes after the angiography, SPECT images were obtained using a rotating, dual-head gamma camera (E. Cam Signature) equipped with low energy, high resolution, and parallel hole collimators. The energy windows were set at 140 keV ± 20% and 90 views were obtained throughout 360° of rotation (128 × 128 matrix, 1.95 mm/pixel) with an acquisition time of 140 s/cycle repeated 6 times. Data processing was performed on a GMS-7700A/EI (Toshiba Medical, Japan). All images were reconstructed using a ramp-filtered back projection and then three-dimensionally smoothed with a Butterworth filter (order 8, cutoff 0.25 cycles/pixel). The reconstructed images were corrected for gamma ray attenuation using the Chang method ($\mu = 0.09$).

2.4. SPECT image analysis using eZIS

We used the eZIS program to supplement the diagnosis and to detect abnormal blood perfusion in each patient. A *Z*-score map for each SPECT image was extracted from the comparison with the mean and standard deviation (SD) of SPECT images of age-matched normal controls that had been incorporated into the eZIS program as a normal control database. A voxel-by-voxel *Z*-score analysis was performed after voxel normalization to global means or cerebellar values; Z-score = ([control mean] – [individual value])/(control SD). These *Z*-score maps were displayed by overlaying them on tomographic sections and projecting the averaged *Z*-score of a 14-mm thickness-to-surface rendering of the anatomically standardized MR imaging template [12].

3. Results

Table 1 summarizes the clinical and demographic characteristics of the NHLE patients associated with autoantibody against the GluR ϵ2. All patients showed various clinical limbic-associated symptoms, such as memory loss, disorientation, hallucination, agitation, delirium, and anxiety. Patients 1 and 2 experienced convulsions during the illness, whereas patients 3, 4, 7, and 8 had generalized seizures at least once at the onset. The electroencephalogram showed a bihemispheric ictal discharge in the bilateral temporal regions in patient 1, and periodic lateralized epileptiform discharges in the left temporal area in patient 2, whereas the other patients had diffusely intermittent slow waves. CSF analysis revealed an increased cell count (normal range: <5 /mm^3) in four patients and increased protein level (normal range: <45 mg/dl) in four patients. Brain MRI showed high signal intensities on T2- and diffusion-weighted images in the bilateral medial temporal lobes, cingulate gyri, and insulae in patient 1 (Fig. 1A), in the right temporal and occipital lobe in patient 2 (Fig. 1B). These lesions showed iso-intensity signals on T1-weighted images and the lack of low apparent diffusion

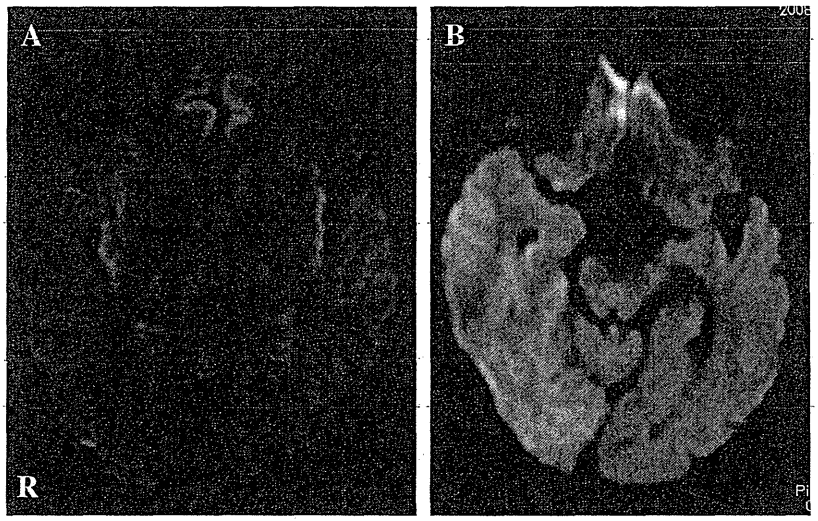

Fig. 1. Diffusion-weighted MR images (axial images) in NHLE patients (patients 1 and 2). Brain MRI shows high signal intensities on diffusion-weighted images in the bilateral medial temporal lobes, cingulate gyri, and insulae in patient 1 (A; cited from reference [21]. Adapted with permission.) and in the right temporal and occipital lobes in patient 2 (B).

coefficient values and gadolinium enhancement. Brain perfusion SPECT was performed in all patients within 20 days (mean duration 10.8 ± 5.8 days) of clinical onset. The eZIS analysis detected significantly increased or decreased regional cerebral blood flow in all patients (Fig. 2, Table 2). Two patients with abnormal MRI findings showed significant hyperperfusion in ipsilateral cerebral cortices. Patient 1 had hyperperfusion in the medial temporal lobes, left putamen, and left insula, and hypoperfusion in the bilateral frontal lobes (Fig. 2A). Patient 2 showed hyperperfusion mainly in the right lateral temporal and occipital lobes and hypoperfusion mainly in

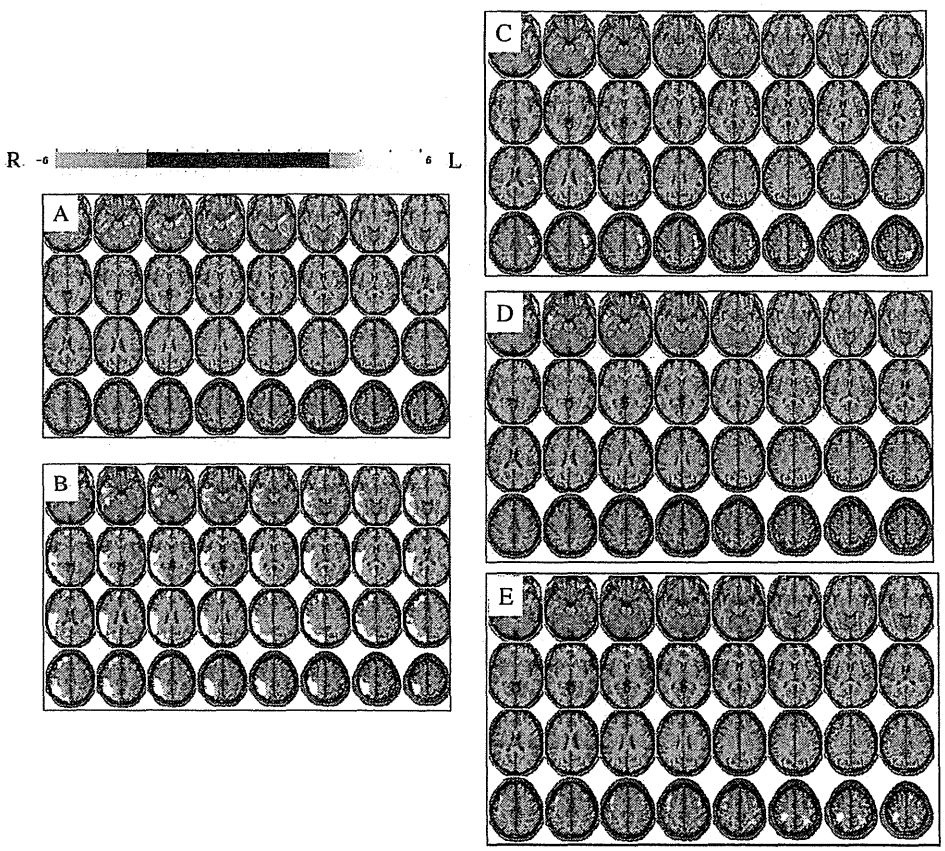

Fig. 2. Easy Z-score imaging system (eZIS) images in patients with NHLE (patients 1, 2, 5, 6, and 7). The color images represent the statistical significance (Z-score) of the increase (red) and decrease (blue) in regional cerebral blood flow. Patient 1 shows hyperperfusion in the bilateral medial temporal lobes, left putamen, and left insula, and hypoperfusion in the bilateral frontal lobes (A; cited from reference [21]. Adapted with permission.). Patient 2 shows hyperperfusion in the right lateral temporal and occipital lobes and hypoperfusion in frontal, parietal, and lateral temporal lobes, insula on the left hemisphere (B). Patients 5 (C), 6 (D), and 7 show hypoperfusion on one or both sides in the limbic system, such as cingulate gyrus, insula, and orbitofrontal cortex, as well as basal ganglia and brainstem (E). Moreover, hyperperfusion was identified in the focal cortical regions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Summary of the SPECT findings.

Patient	SPECT	
	Hyperperfusion areas	Hypoperfusion areas
1	Medial temporal, Lt putamen, and Lt insula	Frontal
2	Rt lateral temporal and Rt occipital	Lt frontal, Lt parietal, Lt lateral temporal, Lt insula, Lt caudate, and cingulate
3	Frontal	Rt medial temporal
4	Rt temporal	Lt insula
5	Lt frontal and Lt temporal	Rt orbitofrontal, Rt isula, Lt putamen, and brainstem
6	Rt lateral temporal	Insula, cingulate, and caudate
7	Frontal and parietal	Rt insula, cingulate, fusiform, and brainstem
8	Frontal and lateral temporal	Fusiform and cerebellum

Rt: right; Lt: left; frontal: frontal lobe; parietal: parietal lobe; lateral temporal: lateral emporal lobe; medial temporal: medial temporal lobe; occipital: occipital lobe; orbitofrontal: orbitofrontal gyrus; cingulate: cingulate gyrus; caudate: caudate nucleus; fusiform: fusiform gyrus.

the insula, caudate nucleus as well as left lateral temporal, parietal, and occipital lobes on the left hemisphere (Fig. 2B). Although the other six patients had normal MRI findings, significant hypoperfusion was identified mainly in one or both sides of the limbic system, such as medial temporal lobe, cingulate gyrus, insula, and orbitofrontal cortex (Fig. 2C–E). In some patients, the regions of hypoperfusion extended to the basal ganglia, brainstem, or cerebellum. Significant hyperperfusion was identified in focal cortical regions and its distribution was different in each patient.

4. Discussion

We examined brain perfusion changes in eight NHLE patients using eZIS analyses. All patients showed typical neurologic symptoms of NHLE and positive autoantibody against the GluR $\epsilon 2$ in cerebrospinal fluid. HSV encephalitis and paraneoplastic limbic encephalitis were excluded by the lack of evidence for HSV infection and systemic malignancies. We suggest that NHLE in our patients may be caused by a transient autoimmune response, rather than by direct viral infection based on the presence of autoantibody.

Previous SPECT imaging studies in patients NHLE associated with the GluR $\epsilon 2$ antibodies showed hyperperfusion in temporal lobe corresponding to the abnormally hyperintense lesions on MRI [6,7]. Our results indicated a significant hyperperfusion that corresponded to hyperintense lesions on diffusion-weighted image and a significant hypoperfusion, predominantly in the limbic system, in the patients without abnormal MRI findings. Two of eight patients showed abnormal hyperintensity in the temporal and occipital lobes, cingulate gyrus, and insula on diffusion-weighted image. These lesions were inconsistent with the ischemic changes because of the normal apparent diffusion coefficient values. The eZIS analysis showed a significant hyperperfusion corresponding to the hyperintense lesions on diffusion-weighted image. These patients developed a convulsive state over the course of the disease. Hyperintense lesions on diffusion-weighted image in convulsive state reflect the cytotoxic edema caused by neuronal excitotoxicity [13,14]. Brain perfusion SPECT in a convulsive state shows compensatory hyperperfusion due to the increased glucose and O_2 required during prolonged overactivation of epileptic neurons. Subsequently, cerebral blood flow is no longer sufficient to prevent local hypoxia, and these events lead to cytotoxic edema and reduced extracellular volume [13,14]. We suggest that hyperintense lesions on diffusion-weighted image with hyperperfusion may be related to not only the inflammatory process itself, but also a prolonged convulsive state.

The most interesting findings in patients without abnormal MRI findings were hypoperfusion predominantly in the medial temporal lobe, cingulate gyrus, insula, and orbitofrontal cortex on eZIS analysis. The limbic system is generally thought to include the hippocampus, amygdale, anterior thalamus, hypothalamus, mammillary bodies, basal forebrain, cingulate gyrus, orbitofrontal cortex, and parahippocampal cortex [15]. Moreover, the insula has a vital role as a limbic integration cortex [16]. These regions are associated with vegetative and survival behaviors, emotions, learning, and memory [17]. Therefore, NHLE patients without abnormal MRI findings showed hypoperfusion predominantly in one or both sides of the limbic and paralimbic areas corresponding to the clinical symptoms. ^{99m}Tc-ECD, which is a lipid soluble tracer, undergoes ester hydrolysis and the acid metabolite is trapped intracellularly for a prolonged period. The distribution of ^{99m}Tc-ECD might reflect not only brain perfusion, but also the reduction of the enzymatic process due to neuronal dysfunction [18–20]. Our results suggest that the ^{99m}Tc-ECD SPECT study may be able to detect neuronal dysfunction in NHLE, even before any morphologic abnormalities become detectable on MRI.

The present study has several limitations. NHLE in our patients may be caused by an undetected infectious agent. The diagnosis was made without examination of other antineuronal antibodies against the anti-NMDA receptor and the anti-VGKCs or pathologic confirmation. The electroencephalogram was not performed at the time of SPECT study. We could not define whether brain perfusion changes specifically related to NHLE or seizure activity, including subclinical seizure. Moreover, the number of patients was small and further studies with larger samples, are needed to confirm our results.

5. Conclusion

NHLE patients associated with GluR $\epsilon 2$ autoantibody showed brain perfusion changes on eZIS analysis. NHLE patients with abnormal MRI findings showed hyperperfusion that corresponded to hyperintense lesions on diffusion-weighted image, whereas those without abnormal MRI findings showed hypoperfusion, predominantly in the limbic and paralimbic areas. We speculate that hyperperfusion in hyperintense lesions reflects compensatory hyperperfusion relating to a prolonged convulsive state, whereas hypoperfusion, predominantly in the limbic system may indicate neuronal dysfunction. We suggest that ^{99m}Tc-ECD SPECT study using eZIS analysis might be helpful to detect the neuronal dysfunction, particularly in NHLE patients without abnormal MRI findings.

Disclosure

Noriyuki Kimura, Toshihide Kumamoto: This study was supported by Health and Labor Science Research Grants for Research on Psychiatry and Neurological Disease and Mental Health (H20-021).

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