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Clinical Short Communication

Detection of LGI1 and CASPR2 antibodies with a commercial cell-based assay in patients with very high VGKC-complex antibody levels

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ABSTRACT

Background: The presence of VGKC-complex antibodies, without LGI1/CASPR2 antibodies, as a standalone marker for neurological autoimmunity remains controversial. Additionally, the lack of an unequivocal VGKC-complex antibody cut-off level defining neurological autoimmunity makes it important to test for monospecific antibodies. We aim to determine the performance characteristics of a commercial assay (Euroimmun, Lübeck, Germany) for LGI1/CASPR2 antibody detection in patients with very high VGKC-complex antibody levels and report their clinico-serological associations.

Methods: We identified 8 patients in our cohort with the highest VGKC-complex antibody levels (median 2663.5 pM, range 933–6730 pM) with VGKC-complex antibody related syndromes (Group A). Two other groups were identified; 1 group with suspected neuronal surface antibody syndromes and negative for VGKC-complex antibodies (Group B, n = 8), and another group with cerebellar ataxia and negative for onconeuronal antibodies (Group C, n = 8).

Results: Seven out of 8 patients (87.5%) in Group A had LGI1 and/or CASPR2 antibodies. One Group B patient had LGI1 antibodies but was negative on re-testing with a live cell assay. No Group C patients had monospecific antibodies. Inter-rater reliability was high; combining Groups A and B patients, the kappa statistic was 0.87 and 1.0 for LGI1 and CASPR2 antibodies respectively.

Conclusion: We demonstrated that a high proportion of patients with very high VGKC-complex antibody levels and relevant clinical syndromes have LGI1 and/or CASPR2 antibodies detected by the commercial assay. Our findings lend support to the use of the assay for rapid and reliable detection of LGI1 and CASPR2 antibodies.

1. Introduction

Antibodies against the voltage-gated potassium channel (VGKC)-complex detected by radioimmunoassay (RIA), especially at high levels (>400 pM), are believed to be supportive of an underlying autoimmune neurological disorder [1,2]. However, the lack of an unequivocal antibody cut-off level defining neurological autoimmunity has led to difficulties with its interpretation [3,4]. Furthermore, the presence of low levels of VGKC-complex antibodies in non-immune-mediated conditions such as Creutzfeldt-Jakob disease and in elderly subjects without neurological disorders, raise questions regarding the relevance of the antibodies in these conditions [5–7]. In 2010, it was demonstrated that the antigenic targets in most patients with high levels of VGKC-complex antibodies were 2 VGKC-complex related proteins: leucine-rich glioma-inactivated protein 1 (LGI1) and contactin-associated protein-like 2 (CASPR2) [8,9]. Patients with antibodies to LGI1 and CASPR2 have well-defined VGKC-complex antibody related syndromes. LGI1 antibodies are associated with limbic encephalitis and facio-brachial dystonic seizures (FBDS) while CASPR2 antibodies are found mostly in patients with neuromyotonia (Isaacs' syndrome) and Morvan's syndrome but can also be present in patients with limbic encephalitis [10].

Testing for LGI1 and CASPR2 antibodies utilizing in-house developed cell-based techniques are available at a few highly specialized laboratories around the world. However, the turn-around time for external requests is usually a few weeks. This may delay diagnosis and preclude immunotherapy in some patients, especially when there are no robust clinical diagnostic criteria. In recent years, commercial cell-based assays have become available allowing for rapid detection of these antibodies and can be performed in clinical laboratories capable of performing indirect immunofluorescence. These assays demonstrate good sensitivity and specificity with reference to the in-house tests performed at highly specialized centers [11]. Herein, we report the performance

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characteristics of a commercial assay for the detection of LGI1 and CASPR2 antibodies in our cohort of patients with very high VGKC-complex antibody levels and their clinico-serological associations.

2. Material and methods

2.1. Patients

Patients tested for VGKC-complex antibodies from January 2013 to October 2015 were identified from the neuroimmunology database at the National Neuroscience Institute, Singapore. For all patients, VGKC-complex antibody RIA was performed at the Oxford Neuroimmunology testing service (Oxford University Hospitals, United Kingdom); seropositivity was defined as VGKC-complex antibody level > 100 pM [8]. Patient information was obtained from review of clinical notes and electronic health records. Forty-two patients were positive for VGKC-complex antibodies during this period (median VGKC-complex antibody level 164.5 pM, range 103–6730 pM). The 8 patients with the highest VGKC-complex antibody levels were identified (median VGKC-complex antibody level 2663.5 pM, range 933–6730 pM) (Group A). All 8 patients presented with well-defined VGKC-complex antibody related syndromes. VGKC-complex antibody related syndromes were defined as limbic encephalitis, FBDS, epilepsy, neuromyotonia (Isaacs' syndrome) and Morvan's syndrome [2,12]. Two other patient groups were selected (Groups B and C). During the period specified above, 372 patients were tested negative for VGKC-complex antibodies, i.e. VGKC-complex antibody level ≤ 100 pM. After exclusion of patients without leftover sera and incomplete clinical data, 8 patients with suspected neuronal surface antibody syndromes (Group B; limbic encephalitis n = 5, epilepsy n = 1, cognitive impairment/dementia n = 2) were identified by 2 authors (T.Y. and Z.C.) based on clinical features, ancillary investigations and response to immunotherapy (if given), guided by criteria proposed by Zuliani et al. [13]. Group B was included as it is recognized that some patients with neuronal surface antibody syndromes have CASPR2 antibodies in the absence of VGKC-complex antibodies [12]. Group C consisted of 8 patients with cerebellar ataxia who were negative for onconeuronal antibodies with stored sera. This group was included as CASPR2 antibodies have been described in a small proportion of patients with idiopathic cerebellar ataxia [14]. All 24 patients recruited in our study have been treated by neurologists from our institution. Sera from Group A and Group B patients used in this study were from the same samples sent to Oxford for VGKC-complex antibody RIA testing. Sera from Group C patients were from the same samples that were tested in our institution for onconeuronal antibodies using a commercial immunoblot assay (Euroline, Euroimmun, Lübeck, Germany).

2.2. Antibody identification

A trained laboratory technologist performed the antibody testing using a commercial indirect immunofluorescence cell-based assay (Autoimmune Encephalitis Mosaic 1, Euroimmun, Lübeck, Germany) according to the manufacturer's recommendations. Each well for a single test sample on the slide consists of 6 biochips containing human embryonic kidney (HEK) 293 cells transfected with neuronal surface protein antigens, namely; LGI1, CASPR2, *N*-methyl-D-aspartate receptor (NMDAR), α-amino-3-hydroxy-5-methyl-4-isoxazol-propionic acid receptor (AMPAR) 1 and 2, and γ-amino-butyric acid receptor ($GABA_BR$). Banked sera stored at −20 °C was thawed and diluted at 1:10. Slides were incubated with the diluted sera, washed and stained with fluorescein-labelled anti-human IgG antibodies. The slides were then visualized using a fluorescence microscope and antibodies were identified by the presence of cytoplasmic and cell membrane immunofluorescence. The results were interpreted qualitatively and independently by 2 neurologists (T.Y. and K.T.) with experience in indirect immunofluorescence interpretation. Blinding of the readers to clinical information and VGKC serostatus was achieved by random sequence sample testing. For an antibody to be identified as present, positive interpretation from both readers were required. Positive and negative controls from the manufacturer were also incubated with the slides during testing. The samples were tested in 2 batches and total time taken was about 5 h.

Our study was approved by the Central Institutional Review Board, Singhealth, Singapore. The commercial assay was provided courtesy of

Table 1
Demographic, clinical and serological features of patients in Group A and Group B.

	Group A (n = 8)	Group B (n = 8)
Age at onset, median (range), years	66 (40–85)	54 (23–82)
Male, No. (%)	5 (62.5)	5 (62.5)
Ethnicity		
Chinese, No. (%)	6 (75)	7 (87.5)
Causacian, No. (%)	1 (12.5)	1 (12.5)
Indian, No. (%)	1 (12.5)	0 (0.0)
Initial presentation		
Seizures, No. (%)	6 (75.0)	4 (50.0)
Cognitive impairment, No. (%)	2 (25.0)	4 (50.0)
Psychiatric symptoms, No. (%)	1 (12.5)	1 (12.5)
Neuromyotonia, No. (%)	1 (12.5)	0 (0.0)
During course of illness		
Seizures, No. (%)	7 (87.5)	5 (62.5)
FBDS, No. (%)	3 (37.5)	0 (0.0)
Cognitive impairment, No. (%)	6 (75.0)	6 (75.0)
Psychiatric symptoms, No. (%)	5 (62.5)	2 (25.0)
Neuromyotonia, No. (%)	1 (12.5)	0 (0.0)
Clinical syndrome		
Limbic encephalitis, No. (%)	5 (62.5)	5 (62.5)
FBDS/Epilepsy, No. (%)	2 (25.0)	1 (0.0)
Cognitive impairment/dementia, No. (%)	0 (0.0)	2 (25.0)
Neuromyotonia, No. (%)	1 (12.5)	0 (0.0)
VGKC-complex antibody level, median (range), pM	2663.5 (933–6730)	Negative
Monospecific antibody		
LGI1 only, No. (%)	4 (50.0)	1 (12.5)
CASPR2 only, No. (%)	2 (25.0)	0 (0.0)
LGI1 and CASPR2, No. (%)	1 (12.5)	0 (0.0)
Hyponatremia, No. (%)	2 (25.0)	0 (0.0)
MRI brain features	n = 8	n = 8
Medial temporal involvement, No. (%)	6 (75.0)	6 (75.0)
Extra-limbic involvement, No. (%)	0 (0.0)	1 (12.5)
Non-specific white matter hyperintensities, No. (%)	1 (12.5)	1 (12.5)
Normal, No. (%)	1 (12.5)	0 (0.0)
EEG features	n = 7	n = 7
Temporal epileptiform activity/electrographic seizures, No. (%)[a]	4 (57.1)	5 (71.4)
Slow activity, No. (%)[a]	1 (14.3)	2 (28.6)
Normal, No. (%)[a]	2 (28.6)	0 (0.0)
CSF features	n = 6	n = 8
Lymphocytic pleocytosis (≥5 cells/μL), No. (%)[b]	0 (0.0)	4 (50.0)
Elevated protein concentration (>0.5 g/L), No. (%)[b]	0 (0.0)	3 (37.5)
Immunotherapy given	n = 6	n = 3
Improvement with immunotherapy, No. (%)[c]	6 (100.0)	2 (66.6)
Tumor, No. (%)[d]	3 (37.5)	0 (0.0)
Nadir mRS, median (range)	2 (1–4)	2.5 (1–6)
Best mRS, median (range)	1 (1–2)	1 (0–6)

All comparative analyses between Groups A and B did not reach statistical significance. No statistically significant change in mRS was observed within Groups A and B.

[a] The percentage was calculated based on the number of patients who had EEG as the denominator.

[b] The percentage was calculated based on the number of patients who had CSF analysis as the denominator.

[c] The percentage was calculated based on the number of patients given immunotherapy as the denominator.

[d] Two patients had rectal/colon adenocarcinoma few months prior to neurological presentation while 1 patient was found to have thymoma during tumor workup after neurological presentation.

Abbreviations: FBDS (facio-brachial dystonic seizures), VGKC (voltage-gated potassium channel), LGI1 (leucine-rich glioma-inactivated protein 1), CASPR2 (contactin-associated protein-like 2), MRI (magnetic resonance imaging), EEG (electroencephalogram), CSF (cerebrospinal fluid), mRS (modified Rankin Scale).