Proposed clinical diagnostic criteria for anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis have high sensitivity and specificity in paediatric patients

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Abstract:

Aim

Determine the validity of the proposed clinical diagnostic criteria for anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in paediatric patients.

Method

The diagnostic criteria for anti-NMDAR encephalitis proposed by Graus et al. use clinical features and conventional investigations to facilitate early immunotherapy before antibody status is available. The criteria are satisfied if patients develop four out of six symptom groups within three months, together with at least one abnormal investigation (electroencephalography/cerebrospinal fluid), and reasonable exclusion of other disorders.

We evaluated the validity of the criteria using a retrospective cohort of paediatric encephalitis patients. Twenty-nine patients with anti-NMDAR encephalitis and 74 encephalitis controls were included.

Results

As expected, the percentage of anti-NMDAR encephalitis patients who fulfilled the clinical criteria increased over time. During the hospital inpatient admission, the majority of patients (26/29, 90%) with anti-NMDAR encephalitis fulfilled the criteria, significantly more than the control group (3/74, 4%) (p=0.0001). The median time of fulfilling the criteria in anti-NMDAR encephalitis patients was 2 weeks from first symptom onset (range 1-6 weeks). The sensitivity of the criteria were 90% (95% confidence intervals 73-98) and the specificity was 96% (95% confidence intervals 89-99).

Interpretation

The proposed diagnostic criteria for anti-NMDAR encephalitis have good sensitivity and specificity. Incomplete criteria do not exclude the diagnosis.

Keywords:

Anti-NMDAR encephalitis; NMDAR antibody; Autoimmune encephalitis; Encephalitis; Neuroimmunology

What this paper adds

- The proposed clinical diagnostic criteria for anti-NMDAR encephalitis by Graus et al have high sensitivity and specificity in paediatric patients.
- The median time of fulfilling the criteria in anti-NMDAR patients was 2 weeks from first symptom onset.

Introduction

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a severe but treatable encephalitis which is increasingly recognized in young individuals. The definite diagnosis of anti-NMDAR encephalitis relies on the demonstration of anti-NMDAR antibodies in cerebrospinal fluid (CSF) in patients with a compatible clinical picture. However, there is often a delay in NMDAR antibody testing, even in resource rich countries. Furthermore, antibody testing is not readily accessible in many parts of the world, particularly resource poor countries. In view of this, Graus et al developed diagnostic criteria for autoimmune encephalitis solely based on neurological assessment and conventional investigations. [1] This approach aimed to allow a prompt 'suspected diagnosis', and allow early initiation of immunotherapy whilst awaiting NMDAR antibody results, with the hope of achieving better clinical outcomes. Our study evaluated the validity of the diagnostic criteria for anti-NMDAR encephalitis in children.

Methods

As per Graus et al, the diagnosis of probable anti-NMDAR encephalitis can be made when all three of the proposed criteria have been met. [1] The first criterion is rapid onset (less than three months) of at least four out of six major groups of clinical symptoms, including (1) abnormal (psychiatric) behavior or cognitive dysfunction, (2) speech dysfunction (pressured speech, verbal reduction, mutism), (3) seizures, (4) movement disorders, dyskinesias, or rigidity/abnormal postures, (5) decreased level of consciousness, and (6) autonomic dysfunction or central hypoventilation. The second criterion is the presence of at least one of the following laboratory study results, either (1) abnormal EEG (focal or diffuse slow or disorganized activity, epileptic activity, or extreme delta brush) or (2) CSF with pleocytosis or oligoclonal bands. The third criterion is reasonable exclusion of other disorders. All three criteria should be fulfilled for a probable diagnosis of anti-NMDAR encephalitis.

The criteria were tested using an established cohort of children (less than 16 years of age) with encephalitis from the Children's Hospital at Westmead, Sydney, Australia.

The clinical features and investigation findings had been retrieved from patients' notes as reported in Pillai et al. [2] In order to increase the cohort of anti-NMDAR encephalitis patients, we also included data on children with anti-NMDAR encephalitis from Starship Children's Hospital, Auckland, New Zealand. [3] The clinical data was retrieved from the case notes by three clinicians in Sydney (ACCH, SSM, SCP), and two clinicians in Auckland (RH, HJ) with consensus agreement on clinical features and timing of acquisition of clinical features. Clinical data in 27 out of 29 anti-NMDAR encephalitis patients were collected before generation and publication of the criteria by Graus et al. The presence of clinical symptoms was reported as positive only when there was clear documentation for at least 24 hours duration. [2] All patients had EEG and CSF studies performed. CSF pleocytosis was defined as CSF white cell count ≥5/uL.

In total, 29 encephalitis patients with anti-NMDAR antibodies detected in CSF or serum (CSF positive = 23, serum only tested = 6) were included in the study. 25 of the 29 anti-NMDAR encephalitis patients received immune therapy during their inpatient admission (steroids n=25, IVIG n=20, plasma exchange n=7, rituximab n=9, cyclophosphamide n=2, other n=2).

To determine the specificity of the criteria, 74 patients with other causes of encephalitis (35 cases of acute disseminated encephalomyelitis (ADEM), 20 cases of enterovirus (EV) encephalitis, 8 cases of herpes simplex virus (HSV) encephalitis and 11 cases of Mycoplasma encephalitis) were used as controls. The HSV encephalitis patients only had a monophasic course and did not have a biphasic course as seen in HSV encephalitis followed by anti-NMDAR encephalitis. 56 of 74 of the encephalitis controls had serum NMDAR antibody testing and were negative. Etiologies of encephalitis were defined according to criteria proposed by Granerod et al and the results were published before commencement of the current study. [2, 4] Cases were classified as confirmed, probable, or possible. Confirmed cases were based on detection of the organism or antibody in CSF or brain. Cases were defined as probable if there was serological evidence of acute infection or antibody production. Possible cases were based on detection of the organism from a specimen sample outside the central nervous system (such as throat, stool, etc). [4] The hierarchical classification for the etiologies and demographics are presented in Table 1.

In order to investigate the validity of the diagnostic criteria during evolution of disease, the criteria were applied to each subject during the hospital inpatient admission (anti-NMDAR encephalitis mean 75 days, median 62, range 11-222, and control encephalitis mean 16 days, median 11, range 1-160). All patients and controls were improving at the time of discharge and had not evolved new clinical problems when seen at outpatient follow-up. The timing (in terms of weeks since first symptom onset) when the criteria were fulfilled (when appropriate) was recorded. The sensitivity and specificity were calculated using three, four and five out of six symptom groups as thresholds. Fisher's exact test was used to compare different variables. The study was approved by the Ethics Committee of The Children's Hospital at Westmead (09/CHW/56) and the Starship Children's Hospital.

Results

As anticipated, the anti-NMDAR encephalitis patients accrued symptoms over time, with 24% fulfilling Graus et al criteria after 1 week of symptoms, 48% after 2 weeks of symptoms (Figure 1a). During the hospital inpatient admission, the median number of symptom domains (maximum 6) present in the anti-NMDAR encephalitis group was 5 (range 3-6), while that in the control group was 2 (range 1-5). Regarding investigations, all patients with anti-NMDAR encephalitis had either an abnormal EEG (25/29, 86%) or an abnormal CSF (25/29, 86%). During the hospital inpatient admission, 26/29 (90%) of the anti-NMDAR encephalitis group, and 3/74 (4%) of the control group fulfilled the Graus et al criteria (p-value=0.0001). The symptom distribution of the 26 anti-NMDAR encephalitis patients who fulfilled the criteria during the hospital inpatient admission is presented in Figure 1b – psychiatric features and movement disorders were the most common, while speech dysfunction and autonomic features were the least common. The three subjects in the anti-NMDAR encephalitis group who did not fulfill the diagnostic criteria were CSF positive for anti-NMDAR antibody. The median time of fulfilling the criteria in anti-NMDAR encephalitis patients was 2 weeks from first symptom onset (range 1-6 weeks) (Figure 1a). The three subjects from the control group who fulfilled the criteria included one patient with confirmed EV encephalitis, and two patients with probable Mycoplasma

encephalitis (all three were confirmed negative for serum anti-NMDAR antibody, brief case descriptions in supplementary material). The sensitivity of the proposed diagnostic criteria during the hospital admission were 90% (95% confidence intervals 73-98%), and the specificity was 96% (95% confidence intervals 89-99%). The sensitivity and specificity of the criteria using three and five symptom groups as cut-offs are presented in Table 2.

Discussion

Our inclusion criteria employed the Granerod encephalitis criteria, as these criteria are applicable to both our anti-NMDAR encephalitis and control encephalitis subgroups. Our study demonstrated that the diagnostic criteria for anti-NMDAR encephalitis proposed by Graus et al have reasonably high sensitivity (90%) and specificity (96%). However, clinicians should be aware that not fulfilling the Graus et al criteria does not exclude the possibility of anti-NMDAR encephalitis, as shown in three of our anti-NMDAR encephalitis who were CSF NMDAR antibody positive but did not fulfill Graus et al criteria. Graus et al noted that clinical suspicion of autoimmune encephalitis in the very young child may be more challenging. Only three of our patients were younger than 2 years of age, and we agree it will be potentially more challenging to identify language, behaviour and autonomic symptoms in such very young pre-verbal children. Moreover, it is worth noting that anti-NMDAR encephalitis is characterized by gradual evolution of symptoms, and the majority of our patients did not satisfy the criteria during the first week of symptoms. In our cohort, the median time of fulfilling the criteria was 2 weeks from first symptom onset (range 1-6 weeks), which is reassuring that using this clinical criteria will not result in significant delay. As evidenced by a large cohort study and a systematic review, early initiation of immune therapy is an independent predictor of good outcome in patients with anti-NMDAR encephalitis. [5, 6] Therefore, the diagnostic criteria are clinically important in guiding treatment in the early stage of disease before antibody status is available.

The practice of NMDAR antibody testing is likely to vary around the world. In some centres with easy and speedy access to NMDAR antibody testing, clinicians will

likely test many patients with unexplained encephalopathy or neuropsychiatric syndromes with a low pre-test probability. By contrast, in resource poor countries, testing may need to be rationalized or may be impossible. In these resource poor countries, the criteria by Graus et al may be highly important, and the sensitivity and specificity resulting from our study are reassuring. We also analyzed how three or five clinical criteria affect the sensitivity and specificity. As anticipated, three clinical criteria had a lower specificity, whereas five clinical criteria had a lower sensitivity. These findings suggest that four clinical criteria is an appropriate cut-off with reasonably high level of both sensitivity and specificity. Although our findings support the utility of the Graus et al criteria as a clinical tool, it is not our recommendation to wait until 4 clinical criteria are fulfilled before testing for NMDAR antibody or treating with immune therapy- instead we would recommend starting immune therapy as soon as autoimmune encephalitis is considered possible.

Subjects with ADEM, EV encephalitis, HSV encephalitis and Mycoplasma encephalitis were selected as controls because they are common causes of infectious and immune-mediated encephalitis in children in our locality (Australia and New Zealand), and they present the most challenging differential diagnoses of anti-NMDAR encephalitis. The three controls who fulfilled Graus et al clinical criteria had confirmed enterovirus encephalitis (n=1) and probable mycoplasma encephalitis (n=2). Although all three were serum NMDAR antibody negative, it would have been useful to test CSF NMDAR antibody, particularly given the previously proposed description of anti-NMDAR encephalitis following mycoplasma infection. [7] Though the sensitivity and specificity in our study were promising, the usefulness of the diagnostic criteria in differentiating anti-NMDAR encephalitis from other causes of infectious encephalitis like Japanese B encephalitis, which is more common in Asia and known to have prominent movement disorders, requires further evaluation. Children with other etiologies of acute encephalopathy such as metabolic, toxic or vascular causes were not recruited as controls since the majority of these acute encephalopathy syndromes can be excluded by careful history and targeted investigations.

The anti-NMDAR encephalitis patients and controls included in our study were

retrieved from our existing database. The clinical symptoms were recorded, and the etiologies of the controls were determined and published in 27 of 29 anti-NMDAR encephalitis patients before designing this study, therefore potentially reducing bias. [2, 3] As might be expected the median and mean duration of inpatient admission was longer for the anti-NMDAR encephalitis patients than the controls, although all individuals were improving at the time of discharge and had not evolved new clinical problems when seen at outpatient follow-up. A limitation of our study was that not all controls were tested for NMDAR antibody, although all controls fulfilled confirmed, probable or possible diagnoses (ADEM, enterovirus etc) using international consensus criteria. [2, 8] Testing of patients with rare encephalitic syndromes or even 'unknown' encephalitis could have also improved our study. We acknowledge that criterion 3 of the Graus et al criteria; 'reasonable exclusion of other disorders', lacks specificity and may differ according to clinical variables, however a list of differential diagnoses of autoimmune encephalitis is available in the supplementary material of the Graus et al paper [1], to guide clinicians in the application of criterion 3.

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Conclusion

The proposed clinical diagnostic criteria for anti-NMDAR encephalitis have high sensitivity and specificity. Paediatricians may use the criteria to guide immunotherapy before the antibody status is available.

Reference

1. Graus F, Titulaer MJ, Balu R, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol* 2016; **15**: 391-404.
2. Pillai SC, Hacohen Y, Tantsis E, et al. Infectious and autoantibody-associated encephalitis: clinical features and long-term outcome. *Pediatrics* 2015; **135**: e974-84.
3. Mohammad SS, Jones H, Hong M, et al. Symptomatic treatment of children with anti-NMDAR encephalitis. *Dev Med Child Neurol* 2016; **58**: 376-84.
4. Granerod J, Cunningham R, Zuckerman M, et al. Causality in acute encephalitis: defining aetiologies. *Epidemiol Infect.* 2010; **138**: 783-800.
5. Titulaer MJ, McCracken L, Gabilondo I, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol* 2013; **12**: 157-65.
6. Nosadini M, Mohammad SS, Ramanathan S, Brilot F, Dale RC. Immune therapy in autoimmune encephalitis: a systematic review. *Expert Rev Neurother* 2015; **15**: 1391-419.
7. Florence NR, Davis RL, Lam C, et al. Anti-N-Methyl-D-Aspartate receptor (NMDAR) encephalitis in children and adolescents. *Ann Neurol* 2009; **66**: 11-18.
8. Krupp LB, Tardieu M, Amato MP, et al. International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. *Mult Scler* 2013; **19**: 1261-7.

Table 1. Anti-NMDAR encephalitis cases and controls. Numbers, hierarchical classification (confirmed, probable, possible) and demographics.

Diagnosis given	Number	Confirmed	Probable	Possible	Gender (M:F)	Age (mean, median, range)
Anti-NMDAR encephalitis	29	23	6	-	12:17	7.3, 6.1 (1.1-16.0)
All controls (ADEM, EV encephalitis, HSV encephalitis, Mycoplasma encephalitis)	74	56	12	6	46:28	6.0, 5.3 (0.2-14.4)
ADEM	35	35	-	-	25:10	6.3, 5.5 (0.4-14.4)
EV encephalitis	20	17	-	3	14: 6	5.4, 3.6 (0.5-13.8)
HSV encephalitis	8	4	1	3	3:5	1.3, 0.7 (0.2-4.2)
Mycoplasma encephalitis	11	-	11	-	4:7	6.7, 6.3 (2.8-13.0)
Total (Anti-NMDAR encephalitis cases + controls)	103	79	18	6	58:45	6.0, 5.3 (0.2-16.0)

ADEM: Acute disseminated encephalomyelitis

EV: Enterovirus

HSV: Herpes simplex virus

Table 2. During the inpatient admission, the numbers of anti-NMDAR encephalitis cases and controls fulfilling the criteria and the sensitivity/specificity using different cut-offs.

Cut-off	Anti-NMDAR encephalitis	Controls	Sensitivity	Specificity
3 clinical symptom groups	29/29 (100%)	29/74 (39%)	100%	61%
4 clinical symptom groups	26/29 (90%)	3/74 (4%)	90%	96%
5 clinical symptom groups	16/29 (55%)	1/74 (1%)	55%	99%

Figure 1a. Cumulative percentage of patients satisfying anti-NMDAR encephalitis diagnostic criteria over time (weeks from first symptom onset).

Figure 1b. Symptom distribution of anti-NMDAR encephalitis patients who satisfied the anti-NMDAR encephalitis diagnostic criteria (n = 26) during the hospital admission.

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Aim

Determine the validity of the proposed clinical diagnostic criteria for anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis in paediatric patients.

Method

The diagnostic criteria for anti-NMDAR encephalitis proposed by Graus et al. use clinical features and conventional investigations to facilitate early immunotherapy before antibody status is available. The criteria are satisfied if patients develop four out of six symptom groups within three months, together with at least one abnormal investigation (electroencephalography/cerebrospinal fluid), and reasonable exclusion of other disorders.

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Interpretation

The proposed diagnostic criteria for anti-NMDAR encephalitis have good sensitivity and specificity. Incomplete criteria do not exclude the diagnosis.

Keywords:

Anti-NMDAR encephalitis; NMDAR antibody; Autoimmune encephalitis; Encephalitis; Neuroimmunology

What this paper adds

- The proposed clinical diagnostic criteria for anti-NMDAR encephalitis by Graus et al have high sensitivity and specificity in paediatric patients.
- The median time of fulfilling the criteria in anti-NMDAR patients was 2 weeks from first symptom onset.

Introduction

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Methods

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In total, 29 encephalitis patients with anti-NMDAR antibodies detected in CSF or serum (CSF positive = 23, serum only tested = 6) were included in the study. 25 of the 29 anti-NMDAR encephalitis patients received immune therapy during their inpatient admission (steroids n=25, IVIG n=20, plasma exchange n=7, rituximab n=9, cyclophosphamide n=2, other n=2).

To determine the specificity of the criteria, 74 patients with other causes of encephalitis (35 cases of acute disseminated encephalomyelitis (ADEM), 20 cases of enterovirus (EV) encephalitis, 8 cases of herpes simplex virus (HSV) encephalitis and 11 cases of Mycoplasma encephalitis) were used as controls. The HSV encephalitis patients only had a monophasic course and did not have a biphasic course as seen in HSV encephalitis followed by anti-NMDAR encephalitis. 56 of 74 of the encephalitis controls had serum NMDAR antibody testing and were negative. Etiologies of encephalitis were defined according to criteria proposed by Granerod et al and the results were published before commencement of the current study. [2, 4] Cases were classified as confirmed, probable, or possible. Confirmed cases were based on detection of the organism or antibody in CSF or brain. Cases were defined as probable if there was serological evidence of acute infection or antibody production. Possible cases were based on detection of the organism from a specimen sample outside the central nervous system (such as throat, stool, etc). [4] The hierarchical classification for the etiologies and demographics are presented in Table 1.

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Results

As anticipated, the anti-NMDAR encephalitis patients accrued symptoms over time, with 24% fulfilling Graus et al criteria after 1 week of symptoms, 48% after 2 weeks of symptoms (Figure 1a). During the hospital inpatient admission, the median number of symptom domains (maximum 6) present in the anti-NMDAR encephalitis group was 5 (range 3-6), while that in the control group was 2 (range 1-5). Regarding investigations, all patients with anti-NMDAR encephalitis had either an abnormal EEG (25/29, 86%) or an abnormal CSF (25/29, 86%). During the hospital inpatient admission, 26/29 (90%) of the anti-NMDAR encephalitis group, and 3/74 (4%) of the control group fulfilled the Graus et al criteria (p-value=0.0001). The symptom distribution of the 26 anti-NMDAR encephalitis patients who fulfilled the criteria during the hospital inpatient admission is presented in Figure 1b – psychiatric features and movement disorders were the most common, while speech dysfunction and autonomic features were the least common. The three subjects in the anti-NMDAR encephalitis group who did not fulfill the diagnostic criteria were CSF positive for anti-NMDAR antibody. The median time of fulfilling the criteria in anti-NMDAR encephalitis patients was 2 weeks from first symptom onset (range 1-6 weeks) (Figure 1a). The three subjects from the control group who fulfilled the criteria included one patient with confirmed EV encephalitis, and two patients with probable Mycoplasma

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Discussion

Our inclusion criteria employed the Granerod encephalitis criteria, as these criteria are applicable to both our anti-NMDAR encephalitis and control encephalitis subgroups. Our study demonstrated that the diagnostic criteria for anti-NMDAR encephalitis proposed by Graus et al have reasonably high sensitivity (90%) and specificity (96%). However, clinicians should be aware that not fulfilling the Graus et al criteria does not exclude the possibility of anti-NMDAR encephalitis, as shown in three of our anti-NMDAR encephalitis who were CSF NMDAR antibody positive but did not fulfill Graus et al criteria. Graus et al noted that clinical suspicion of autoimmune encephalitis in the very young child may be more challenging. Only three of our patients were younger than 2 years of age, and we agree it will be potentially more challenging to identify language, behaviour and autonomic symptoms in such very young pre-verbal children. Moreover, it is worth noting that anti-NMDAR encephalitis is characterized by gradual evolution of symptoms, and the majority of our patients did not satisfy the criteria during the first week of symptoms. In our cohort, the median time of fulfilling the criteria was 2 weeks from first symptom onset (range 1-6 weeks), which is reassuring that using this clinical criteria will not result in significant delay. As evidenced by a large cohort study and a systematic review, early initiation of immune therapy is an independent predictor of good outcome in patients with anti-NMDAR encephalitis. [5, 6] Therefore, the diagnostic criteria are clinically important in guiding treatment in the early stage of disease before antibody status is available.

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likely test many patients with unexplained encephalopathy or neuropsychiatric syndromes with a low pre-test probability. By contrast, in resource poor countries, testing may need to be rationalized or may be impossible. In these resource poor countries, the criteria by Graus et al may be highly important, and the sensitivity and specificity resulting from our study are reassuring. We also analyzed how three or five clinical criteria affect the sensitivity and specificity. As anticipated, three clinical criteria had a lower specificity, whereas five clinical criteria had a lower sensitivity. These findings suggest that four clinical criteria is an appropriate cut-off with reasonably high level of both sensitivity and specificity. Although our findings support the utility of the Graus et al criteria as a clinical tool, it is not our recommendation to wait until 4 clinical criteria are fulfilled before testing for NMDAR antibody or treating with immune therapy- instead we would recommend starting immune therapy as soon as autoimmune encephalitis is considered possible.

Subjects with ADEM, EV encephalitis, HSV encephalitis and Mycoplasma encephalitis were selected as controls because they are common causes of infectious and immune-mediated encephalitis in children in our locality (Australia and New Zealand), and they present the most challenging differential diagnoses of anti-NMDAR encephalitis. The three controls who fulfilled Graus et al clinical criteria had confirmed enterovirus encephalitis (n=1) and probable mycoplasma encephalitis (n=2). Although all three were serum NMDAR antibody negative, it would have been useful to test CSF NMDAR antibody, particularly given the previously proposed description of anti-NMDAR encephalitis following mycoplasma infection. [7] Though the sensitivity and specificity in our study were promising, the usefulness of the diagnostic criteria in differentiating anti-NMDAR encephalitis from other causes of infectious encephalitis like Japanese B encephalitis, which is more common in Asia and known to have prominent movement disorders, requires further evaluation. Children with other etiologies of acute encephalopathy such as metabolic, toxic or vascular causes were not recruited as controls since the majority of these acute encephalopathy syndromes can be excluded by careful history and targeted investigations.

The anti-NMDAR encephalitis patients and controls included in our study were

retrieved from our existing database. The clinical symptoms were recorded, and the etiologies of the controls were determined and published in 27 of 29 anti-NMDAR encephalitis patients before designing this study, therefore potentially reducing bias. [2, 3] As might be expected the median and mean duration of inpatient admission was longer for the anti-NMDAR encephalitis patients than the controls, although all individuals were improving at the time of discharge and had not evolved new clinical problems when seen at outpatient follow-up. A limitation of our study was that not all controls were tested for NMDAR antibody, although all controls fulfilled confirmed, probable or possible diagnoses (ADEM, enterovirus etc) using international consensus criteria. [2, 8] Testing of patients with rare encephalitic syndromes or even 'unknown' encephalitis could have also improved our study. We acknowledge that criterion 3 of the Graus et al criteria; 'reasonable exclusion of other disorders', lacks specificity and may differ according to clinical variables, however a list of differential diagnoses of autoimmune encephalitis is available in the supplementary material of the Graus et al paper [1], to guide clinicians in the application of criterion 3.

Conclusion

The proposed clinical diagnostic criteria for anti-NMDAR encephalitis have high sensitivity and specificity. Paediatricians may use the criteria to guide immunotherapy before the antibody status is available.

Reference

1. Graus F, Titulaer MJ, Balu R, et al. A clinical approach to diagnosis of autoimmune encephalitis. *Lancet Neurol* 2016; **15**: 391-404.
2. Pillai SC, Hacohen Y, Tantsis E, et al. Infectious and autoantibody-associated encephalitis: clinical features and long-term outcome. *Pediatrics* 2015; **135**: e974-84.
3. Mohammad SS, Jones H, Hong M, et al. Symptomatic treatment of children with anti-NMDAR encephalitis. *Dev Med Child Neurol* 2016; **58**: 376-84.
4. Granerod J, Cunningham R, Zuckerman M, et al. Causality in acute encephalitis: defining aetiologies. *Epidemiol Infect.* 2010; **138**: 783-800.
5. Titulaer MJ, McCracken L, Gabilondo I, et al. Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study. *Lancet Neurol* 2013; **12**: 157-65.
6. Nosadini M, Mohammad SS, Ramanathan S, Brilot F, Dale RC. Immune therapy in autoimmune encephalitis: a systematic review. *Expert Rev Neurother* 2015; **15**: 1391-419.
7. Florence NR, Davis RL, Lam C, et al. Anti-N-Methyl-D-Aspartate receptor (NMDAR) encephalitis in children and adolescents. *Ann Neurol* 2009; **66**: 11-18.
8. Krupp LB, Tardieu M, Amato MP, et al. International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. *Mult Scler* 2013; **19**: 1261-7.

Table 1. Anti-NMDAR encephalitis cases and controls. Numbers, hierarchical classification (confirmed, probable, possible) and demographics.

Diagnosis given	Number	Confirmed	Probable	Possible	Gender (M:F)	Age (mean, median, range)
Anti-NMDAR encephalitis	29	23	6	-	12:17	7.3, 6.1 (1.1-16.0)
All controls (ADEM, EV encephalitis, HSV encephalitis, Mycoplasma encephalitis)	74	56	12	6	46:28	6.0, 5.3 (0.2-14.4)
ADEM	35	35	-	-	25:10	6.3, 5.5 (0.4-14.4)
EV encephalitis	20	17	-	3	14: 6	5.4, 3.6 (0.5-13.8)
HSV encephalitis	8	4	1	3	3:5	1.3, 0.7 (0.2-4.2)
Mycoplasma encephalitis	11	-	11	-	4:7	6.7, 6.3 (2.8-13.0)
Total (Anti-NMDAR encephalitis cases + controls)	103	79	18	6	58:45	6.0, 5.3 (0.2-16.0)

ADEM: Acute disseminated encephalomyelitis

EV: Enterovirus

HSV: Herpes simplex virus

Table 2. During the inpatient admission, the numbers of anti-NMDAR encephalitis cases and controls fulfilling the criteria and the sensitivity/specificity using different cut-offs.

Cut-off	Anti-NMDAR encephalitis	Controls	Sensitivity	Specificity
3 clinical symptom groups	29/29 (100%)	29/74 (39%)	100%	61%
4 clinical symptom groups	26/29 (90%)	3/74 (4%)	90%	96%
5 clinical symptom groups	16/29 (55%)	1/74 (1%)	55%	99%

Figure 1a. Cumulative percentage of patients satisfying anti-NMDAR encephalitis diagnostic criteria over time (weeks from first symptom onset).

Figure 1b. Symptom distribution of anti-NMDAR encephalitis patients who satisfied the anti-NMDAR encephalitis diagnostic criteria (n = 26) during the hospital admission.

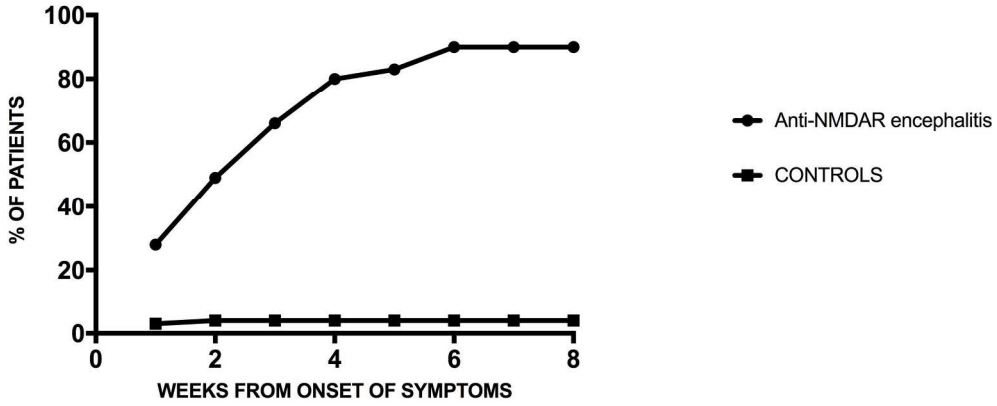

Figure 1a

886x376mm (72 x 72 DPI)

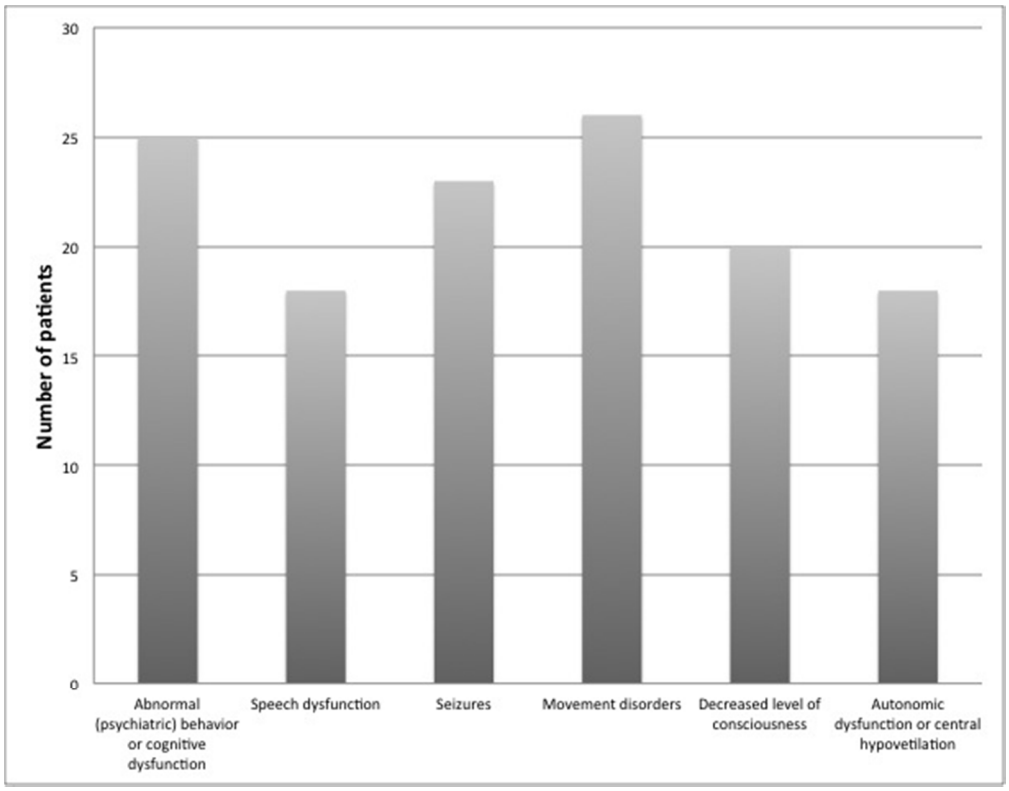

Figure 1b

215x168mm (72 x 72 DPI)

Encephalitis controls that fulfilled Graus et al suspected anti-NMDAR encephalitis criteria.

A three and a half year old previously well male presented with a rapidly progressive encephalopathy requiring hospital admission. He had had a viral upper respiratory tract infection in the week before neurological symptoms. During the first week, the patient was drowsy, behaviourally altered and agitated, had asymmetrical limb and neck dystonic and athetoid movements, and autonomic symptoms (hypertension and diaphoresis otherwise not explained). EEG showed encephalopathic slowing and CSF showed no pleocytosis but was positive for enterovirus PCR. MRI showed inflammatory lesions of the white matter. The patient improved quickly over the following 3 weeks and was discharged after a 21 day admission. He has been followed for 58 months and has been left with some mild inattention and mild cognitive problems. The patient had 4 clinical features using Graus et al criteria: decreased level of consciousness, agitation, movement disorder and autonomic features.

A 4 year old previously well female presented with a rapidly evolving encephalitis, with decreased consciousness, agitation, loss of speech, movement disorder and seizures within the first week of symptom onset occurring after a febrile illness with respiratory symptoms. The syndrome evolved to a complex movement disorder with stimulus sensitive myoclonus, dystonia, and a refractory seizure disorder with focal seizures and status epilepticus requiring intravenous midazolam infusion and intensive care admission for 70 days. CSF revealed 18 white cells (8 monocytes, 10 polymorphs), raised CSF neopterin (871, normal<30), his EEG showed encephalopathy and epileptic activity, yet MRI was normal. NMDAR antibody in serum was negative, as were glycine receptor antibody, GAD antibody, and Voltage gated potassium channel antibody. Mycoplasma IgM was positive. The clinical impression was suspected autoimmune encephalitis, yet treatment with intravenous steroids, intravenous immunoglobulin and rituximab had little impact, and after a 160 day admission, the patient was discharged to rehabilitation, and 24 months later had ongoing significant motor and cognitive disability, and refractory epilepsy. The patient had 5 Graus et al clinical features: decreased consciousness, speech loss, agitation and behavioural change, movement disorder and seizure disorder.

A 2.8 year old previously well female presented after a febrile illness with respiratory symptoms and became confused, irritable, agitated and had decreased consciousness. During admission to hospital, there was loss of speech, ongoing encephalopathy, agitation and autonomic features (hypertension and tachycardia not otherwise explained) and cerebellar signs. MRI showed cerebellar inflammatory features, CSF revealed a pleocytsosis of 20 cells/mm3 (5 monocytes, 15 polymorphs). Mycoplasma IgM was positive. There was a 17 day admission, but the patient made a good recovery duration inpatient stay and was normal at 2 month follow-up. The patient had 4 Graus et al clinical criteria: decreased consciousness, agitation and behavioural change, speech loss and autonomic features.