ORIGINAL ARTICLE

Retrospective study of N-methyl-D-aspartate glutamate receptor IgG testing outcomes at LabPLUS, Auckland City Hospital, New Zealand, 2015 – 2020, in a clinically demand-managed setting

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ABSTRACT

Objectives: To review the outcomes of N-methyl-D-aspartate glutamate receptor (NMDAR) antibody testing conducted at LabPLUS over the period 2015-2020.

Methods: A data extract over the period was obtained detailing patient testing. Retrospectively, a proportion of the tested patient cohort had their clinical records accessed to determine their final diagnoses.

Results: 654 patients were tested which resulted in the identification of 24 NMDAR antibody positive patients. All positive patients met a case definition for NMDAR encephalitis. A review of 100 NMDAR antibody negative patients did not identify any that supported a case definition for NMDAR encephalitis. The neurologist vetted cohort was predominantly adult (80%) with higher numbers of females. Patients of both genders ≤45 years of age accounted for 88% of the antibody positive cases. There was generally high compliance (>75%) in obtaining CSF specimens for testing from within the NRA (local testing) region which was critical, as 30% of patients would have had a delayed diagnosis and most likely treatment if an independent serum specimen had been sent. The laboratory was able to consistently meet the clinically required KPI of same working day reporting turn around times over the six year period for cases of suspected NMDAR encephalitis.

Conclusions: Under the framework of neurologist gate keeping and liaison LabPLUS was able to implement and consistently deliver high value clinical results (antibody positive and negative) that allowed rapid treatment intervention when indicated which then translated into the best possible outcomes for patients with NMDAR encephalitis. The success of the mono-specific NMDAR antibody service allowed further neuroimmunology assays to be added to the diagnostic portfolio.

Key words: N-methyl-D-aspartate glutamate receptor, encephalitis, clinically-managed service, turn around time.

N Z J Med Lab Sci 2021; 75: 192-201

INTRODUCTION

Anti-NMDAR (N-methyl-D-aspartate glutamate receptor) encephalitis is an autoimmune based syndrome with a progressive clinical course that can be treated. The syndrome affects females in a ratio of 4:1 over males, can occur at any age but peak incidence is seen in children and young adults (1). In approximately 60% of females with the disease aged 17 years or more, there is an underlying tumour (often unidentified) with ovarian teratoma being the commonest (2). The clinical presentation can be broken down into four phases:

1. Prodromal phase

In approximately 70% of patients the encephalitis commences with somewhat non-specific symptoms of fever, malaise, poor concentration, headaches, nausea, vomiting, and diarrhoea. This phase lasts from one to three weeks (3). The remaining phases can vary both in terms of sequence and severity.

2. Psychotic and / or seizure phase

Patients undergo behavioural and emotional changes including fear, apathy, depression, decreased cognitive skills, and psychosis (4). Ataxia and choreiform movements may be noted (5). Seizures (tonic-clonic) may also occur; the severity and management of which may require admission to intensive care facilities where monitored settings for cardiac and respiratory support are available.

3. Unresponsive phase

Patients are unable to follow verbal commands and may appear mute. Other symptoms can include maintaining gaze as if in a catatonic state, smiling inappropriately and stereotyped athetotic movements of the hands and fingers (6).

4. Hyperkinetic phase

This phase is characterised by autonomic instability manifesting with cardiac arrhythmia, hypotension, hypertension, hyperventilation, hyperthermia and hypothermia. Dyskinesias, extra-pyramidal signs and stereotyped motor automatisms (lip smacking, teeth clenching, grimacing, sustained jaw movements, and oculogyric crisis) may be observed (7). Autonomic dysregulation is more common in adults, whereas speech dysfunction is more common in children (4).

Once the diagnosis of anti-NMDAR encephalitis is confirmed, aggressive treatment using high dose corticosteroids, immunosuppression, intravenous immunoglobulin, plasma exchange and rituximab either sequentially or in combination is given. Where identified, resection of the tumour is performed (6).

With prompt aggressive treatment the prognosis is good with approximately 75% of affected patients experiencing complete or near complete recovery (3). In the setting of delayed or ineffective treatment, mortality rates can exceed 90% (1). Relapses can occur in 20-25% of patients at intervals ranging from three months to nine years after the initial presentation (8). The commonest symptoms of relapsing patients include speech dysfunction, psychiatric symptoms, seizures, and disturbances of consciousness and attention. Relapse rates are highest in patients not treated with immunotherapeutics in the first episode and those where tumour resection was not performed (3,9).

Prior to 2015 all neuronal antibody requests received at LabPLUS were sent overseas to multiple institutions, many of which were tested in research laboratories rather than accredited diagnostic laboratories. There was no clinical or technical oversight in the send away process leading to

inappropriate requesting, high service costs, and result turn around times (TAT) that exceeded the clinical relevance. It was against this backdrop that a planned on-site (LabPLUS) diagnostic neuroimmunology service was envisioned. The new service was demand-managed by neurologists acting as gatekeepers for all requested tests. Immunopathologists added value in discussions that were required for challenging cases as well as providing an interface for the diagnostic laboratory. For reasons outlined earlier in this introduction, NMDAR encephalitis was the first condition chosen to deliver on-site testing, not only to improve patient outcomes but also to demonstrate proof of concept of the new service which, if delivered, would then allow future expansion.

The Euroimmun Glutamate Receptor 3 Indirect Immunofluorescent (IIF) assay was initially verified for use by on-site (LabPLUS) testing of retained specimens (serum or CSF; N=35) that had been referred to Royal Brisbane Hospital, Australia or Oxford Hospital Laboratories, UK. Concordance with the overseas testing site was 100% (14 positive, 21 negative; unpublished data).

Following the verification, a 10-month pre-and post-implementation audit was conducted (98 patients pre-implementation; 106 patients post-implementation). The three main findings [unpublished data] were:

1. A significant decrease in result TAT from 25 days overseas to three days on-site.
2. Reduction of inappropriate requests as a direct result of the demand-managed process. The most significant change was seen from the Child and Family Unit, ACH where in the pre-implementation period 46 tests were sent overseas compared with four tests performed on site in the post-implementation audit period (91% decrease).
3. A significant improvement in taking the clinically appropriate specimen type. Pre-implementation CSF with or without serum: 36/114 – 31%; post-implementation CSF with or without serum 110 / 150 – 73%.

This retrospective review study was initiated to (a) determine if the improvements seen immediately after implementation had been retained consistently over time (six years); (b) to characterise our testing population on the basis of gender, age and site of referral; and (c) to establish the assay's overall performance in terms of both definitive diagnosis and exclusion utility.

MATERIALS AND METHODS

Euroimmun Glutamate Receptor 3 IIF assay

The principle of the assay is IIF where per microscope field (patient test) there are both transfected (NR1 subunit of the NMDAR complex in EU90 cells) and untransfected EU90 cells embedded. After initial specimen incubation and wash steps a goat anti-human IgG (ɣ-chain specific) –FITC reagent is added. After a further set of incubation and wash steps slides had cover slips applied and were viewed for characteristic (nuclear with cytoplasmic extensions) staining using Zeiss LED-based fluorescence microscopy (excitation: 470/40nm; emission: 515nm) at a magnification of X200 (Figure 1). Serum specimens were tested at dilutions of 1:10 and 1:50. CSF specimens were tested undiluted and at a dilution of 1:10. Phosphate-buffered saline was used as the specimen diluent.

Results are typically reported qualitatively (detected/not detected). However, the analytical and reporting system is sufficiently flexible to accommodate semi-quantitative testing and reporting in challenging cases.

Data extraction

Review period: 1 January 2015 – 6 November 2020. Note: year 2020 comprised 10 months data and testing numbers were impacted by the COVID-19 global pandemic.

Regional classification: Northern Regional Alliance (NRA): ADHB (Auckland City Hospital, Starship Children's Hospital), CMDHB (Middlemore Hospital), WMDHB (North Shore Hospital), NDHB (Whangarei Base Hospital, Dargaville Hospital, Bay of Islands Hospital, Kaitaia Hospital). Patients were identified as being within or having specimens referred for testing from outside the NRA.

Paediatric/adult age classification: Patients ≤ 15 years of age were classified as paediatric.; patients ≥ 16 years of age were classified as adult.

Patient diagnoses in NMDAR antibody positive (N=11) and negative cohorts (N=100) from within the NRA region: Diagnoses were determined by clinical chart review of medical records. Patient identities were not disclosed and data was used anonymously. As this was a retrospective study with no modification on clinical decision making or individual follow up, patient informed consent was not required.

RESULTS

Overview

In the review period, 654 patients underwent testing for mono-specific NMDAR encephalitis, averaging 109 patients per annum. Of the 654 patients tested, 24 cases of NMDAR encephalitis were identified (3.7%); range: 1.9% - 6.3%), positive cases being seen in every year testing was conducted (Figure 2). Of the 24 positive cases, 14 (58%) were female and 10 were male (42%). A single patient had confirmed NMDAR encephalitis historically and was admitted to hospital for on-going seizures, the remaining 23 cases were new diagnoses.

Further, in February 2017, the limbic encephalitis service was expanded (in addition to NMDAR antibody) to include the following five targets in a single test format: (a) leucine-rich glioma inactivated protein (LGI 1), (b) α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA 1/AMPA 2), (c) B1 subunit of the ɣ-aminobutyric acid receptor ($GABA_B$), and (d) contactin associated protein 2 (CASPR 2). The testing is commonly referred to as the 'LE chip'.

A full review of testing outcomes from the LE chip will be presented in a subsequent paper. However, with direct reference to NMDAR antibodies, in the review period, 693 patients were tested using the LE chip with a further 3 positive cases identified (0.4%). With the inclusion of the LE chip testing, there were 27 positive cases out of 1,347 patients tested (2.0%). The data presented in this retrospective review is restricted to those patients whose specimens underwent testing using the mono-specific NMDAR antibody assay.

Patient age/gender characterisation

In every year, numbers of female patients exceeded males by an average of 1.4:1 across the six years. The average did not differ markedly from year to year (range: 1.2:1 – 1.6:1) (Table 1). Patients were predominantly adult across both genders (80% adult, 20% paediatric) over the 6 year period with little variation seen year to year (Table 1). Almost one-quarter (24%) of males and females were in the highest age size class (>61 years) (Table 1). The majority of NMDAR IgG positive cases were in patients ≤ 45 years of age (87.5%), with 62.5% of cases aged ≤ 30 years (Figure 3). In the same age bracket (0-30 years) patients tested accounted for 43.7% (286 / 654) of the total (Table 1, Figure 3).

There were approximately equal numbers of male and female NMDAR IgG positive paediatric cases (Table 2). There were over twice as many adult antibody positive cases with a higher (almost doubled) female predominance (11 F, 6 M) (Table 2). For antibody positive adult males, they were either in a relatively young age group (range: 22-27 years) or in older age group (range: 64-86 years) (Table 2). For antibody positive adult females, all cases were in a relatively tight age range of 18-42 years with a mode of 31 years (Table 2).

Specimen type site/referral

Approximately twice the numbers of paediatric patients were tested from within the NRA as opposed to outside the region and this was consistent year to year. Conversely, higher numbers of adults were referred for testing from outside the NRA at an overall ratio of 1.75:1 across the six years (Figure 4). It was noted that for the initial two years, referred adult specimen numbers from within and outside the NRA were approximately equal. However, over the period 2017 – 2019 external NRA referred specimen numbers were consistently higher (minimum three times) compared to those sent for testing from within the NRA.

Whether referred from within or external to the NRA there was very good compliance of taking a CSF specimen type (independently or with a paired serum) for investigations on paediatric patients which was consistent over the six year period (NRA – 68/89; average: 76%), External to NRA – 32/41 (average: 78%) (Figure 4). However, for adult patients from within the NRA, over the six year period while there was acceptable compliance in obtaining CSF specimens (133/190 (average: 70%: range: 54% - 82%), outside the NRA the overall frequency of taking a CSF specimen dropped to a low average of 49% (163/334) with a range of 30% - 57% (Figure 4).

Twenty-one of the twenty-four (87.5%) NMDAR IgG positive cases had a referred CSF (N=3) or paired CSF and serum (N=18) (Table 3). Of the 18 patients where a CSF and serum was collected, at time of testing, antibody reactivity was only seen in the CSF specimen in seven of the cases (39%).

Report turnaround time (TAT)

In this study, TAT is defined as the time (days) from specimen receipt to the issuing (electronic) of the report. For all NMDAR IgG antibody positive cases the requesting clinician was directly contacted on the day the report was issued. For the 24 antibody positive cases, the average TAT was 0.9 days with a range of 0 -2 days. Cases within the NRA (N=11) had a faster average TAT of 0.5 days (range 0-1 day) compared to those referred from outside the region (N=13; average; 1.1 day, range 0-2 days) (Table 3).

Confirmation of NMDAR encephalitis in an antibody positive setting

For the 23 patients with an undiagnosed acute encephalopathic picture that were NMDAR antibody positive, a formal diagnosis of NMDAR encephalitis was confirmed. Of the 23 new cases of NMDAR encephalitis, 10 were within the NRA region, seven being adult and three paediatric. The clinical records of all 10 cases were reviewed as follows:

For the adult cases, all seven patients had serum and CSF specimens taken and a single case (7) had isolated reactivity in the CSF specimen (15%). All patients had an acute/sub-acute (< three months) presentation with compatible limbic encephalopathic features (Table 4). A single patient (1/6; 17%) had an abnormal MRI, and an additional patient (1/2; 50%) had an abnormal EEG (Table 4). Elevations in either CSF white cell counts and/or protein levels were seen in 5/7 patients (71%) (Table 4). Two of the five (40%) female patients had ovarian tumours which were resected. All patients were treated with combinations of immunosuppressive drugs, IVIG, PLEX and Rituximab, and all demonstrated improved clinical outcomes (Table 4).

A single paediatric case was a patient who had a severe clinical course of NMDAR encephalitis nine years previously, and although rapid treatment was afforded significant developmental retardation and physical (foot and spine) deformities were secondary to the encephalopathy (9). The review was for an increasing frequency of seizures. Although NMDAR antibody was present in the patient's CSF there were no other clinical features or investigations that supported a flare or recurrence of the NMDAR encephalopathy. The seizures were controlled by increasing the dose of Valproate (Table 4).

For the remaining three new paediatric cases, two had serum and CSF specimens taken which were antibody reactive in both specimen types. The third patient's diagnosis was delayed due to parental non-consent for a LP procedure (11). For this patient two serum tests were negative for the antibody before seroconverting on a third bleed (11). All three patients had an acute/sub-acute (< 3 months) presentation with compatible limbic encephalopathic features (Table 4). No abnormalities were seen on MRI (2/2 patients); however, EEG abnormalities were seen in all cases (Table 4). Elevations in CSF white cell counts were seen in 2/2 patients (100%) (Table 4). All patients were treated with combinations of immunosuppressive drugs, IVIG, and Rituximab, and all demonstrated improved clinical outcomes (Table 4).

Table 1. Age and gender distribution of 654 patients tested at LabPLUS for NMDAR IgG 2015 – 2020.

	Male						Female					
	0-15	16-30	31-45	46-60	61+	Totals	0-15	16-30	31-45	46-60	61+	Totals
2015	4	6	1	13	10	34	10	16	11	8	6	51
2016	7	18	6	10	21	62	11	15	12	19	22	79
2017	13	11	9	8	23	64	16	11	19	13	16	75
2018	9	12	3	7	9	40	16	19	13	8	18	64
2019	10	12	2	7	10	41	10	14	14	12	15	65
2020	9	9	3	4	8	33	15	13	6	6	6	46
Totals	52	68	24	49	81	274	78	88	75	66	73	380

Exclusion of NMDAR encephalitis in an antibody negative result setting

Clinical records of 100 (37%) of the 269 NRA NMDAR antibody negative patients were reviewed. The proportion of adult to paediatric cases reviewed matched the frequency of that seen in the NMDAR antibody positive cases (i.e. 70% adult; 30% paediatric). The overall frequency of female (F) to male (M) records reviewed was 2:1, broadly in-line with that seen in the total review cohort of 654 patients. For the adult group the F:M ratio of records reviewed was 1.9:1. For the paediatric group, the F: M ratio of records reviewed was 2.3:1. Overall, 75% of the patients reviewed had a CSF specimen taken (adult N=50/70; 71%); paediatric (N= 25/30; 83%). None of the 25 patients who had a serum specimen only tested had any subsequent specimens taken for analysis.

The largest group (N=16; 16%) of patients were those that had an acute psychotic episode/hallucinations (Figure 5). Within this group approximately equal numbers had CSF specimens (N=9) versus independent serum specimens (N=7) provided for testing. Of those patients with an encephalopathic clinical presentation supported by imaging findings (N=9; 9%), six (67%) patients had either an autoimmune basis (N=4) or an infectious disease basis (N=2). For those patients with an autoimmune basis two were a limbic encephalopathy (LGI1 and GABA-B antibody mediated), one was NMOSD (MOG antibody mediated), and one was due to Hashimoto's thyroiditis (autoimmune mediated hypothyroidism).

Table 2. Gender and age characterisation of the 24 patients tested positive for NMDAR IgG at LabPLUS 2015 – 2020.

Paediatric (N=7)		Adult (N=17)	
Gender	**Age**	**Gender**	**Age**
Male	3	Male	66
Male	7	Male	86
Male	11	Female	18
Female	1	Female	18
Female	13	Female	19
Female	14	Female	21
Female	14	Female	30
Adult (N=17)		Female	31
Gender	**Age**	Female	31
Male	22	Female	32
Male	23	Female	37
Male	27	Female	38
Male	64	Female	42

Table 3. Referral site, Age group, Specimen type and reporting turn-around time [TAT] for 23 patients tested as NMDAR IgG positive at LabPLUS 2015 – 2020.

Patient	Referral site	Paediatric/ Adult	Specimen type	TAT (days)	Patient	Referral site	Paediatric/ Adult	Specimen type	TAT (days)
1	External	Adult	CSF	2	**13**	External	Adult	Serum and CSF	2
2	External	Adult	Serum	2	**14**	NRA	Paediatric	Serum and CSF	0
3	External	Adult	Serum	1	**15**	NRA	Adult	Serum and CSF	0
4	External	Adult	Serum and CSF	1	**16**	NRA	Paediatric	Serum and CSF	1
5	External	Adult	Serum and CSF	0	**17**	External	Paediatric	Serum and CSF	1
6	NRA	Adult	Serum and CSF	1	**18**	External	Paediatric	Serum and CSF	2
7	NRA	Adult	Serum and CSF	1	**19**	NRA	Adult	Serum and CSF	0
8	NRA	Adult	Serum and CSF	0	**20**	External	Adult	Serum and CSF	1
9	External	Adult	Serum and CSF	2	**21**	NRA	Paediatric	Serum and CSF	1
10	External	Adult	Serum and CSF	0	**22**	NRA	Paediatric	Serum and CSF	1
11	NRA	Adult	Serum and CSF	1	**23**	External	Paediatric	CSF	1
12	External	Adult	CSF	0	**24**	NRA	Adult	Serum and CSF	0

TAT: Specimen received to report issued.

Table 4. Clinical features, diagnostic investigations, treatments and outcomes of the 11 patients within the NRA region who were NMDAR antibody positive.

Case	Adult/ Paediatric	Gender	NMDAR antibody	Symptom onset	Clinical features	MRI	EEG	CSF WCC (>5mm^2) Protein (>0.45g/L)	CT	Treatment	Outcome
1	Adult	Female	Serum & CSF both positive	1 week	Confusion, psychoses, catatonia, agitation	Normal	Not performed	WCC: 7	No ovarian teratoma	Immunosuppression IVIG	Improved but ongoing cognitive impairment
2	Adult	Female	Serum & CSF both positive	2 weeks	Confusion, paranoia, respiratory failure, movement disorder	Normal	Normal	Protein: 0.47	No ovarian teratoma	IVIG Rituximab	Improved but secondary neurologic symptoms manifest
3	Adult	Male	Serum & CSF both positive	3 days	Confusion, behavioural changes, agitation	Consistent with encephalopathy	Not performed	WCC: 11	Not performed	Immunosuppression Rituximab	Fully recovered
4	Adult	Female	Serum & CSF both positive	2 weeks	Psychoses, catatonia, agitation	Normal	Not performed	Normal	No ovarian teratoma	Immunosuppression VIG PLEX Rituximab	Fully recovered
5	Adult	Male	Serum & CSF both positive	1 month	Cognitive impairment, ID co-morbidity	Normal	Not performed	Normal	Not performed	Immunosuppression IVIG Rituximab	Significant cognition improvement
6	Adult	Female	Serum & CSF both positive	1 week	Headaches, seizures, hallucinations	Not completed	Abnormal consistent with encephalopathy	WCC: 94	Ovarian teratoma present: resected	Immunosuppression IVIG Rituximab	Fully recovered
7	Adult	Female	Serum & CSF tested. CSF only positive	1 month	Hallucinations. Dysphagia, behavioural changes	Normal	Not performed	WCC: 37	Ovarian teratoma present: resected	Immunosuppression IVIG	Fully recovered
Case	**Adult / Paediatric**	**Gender**	**NMDAR antibody**	**Symptom onset**	**Clinical features**	**MRI**	**EEG**	**CSF WCC (>5mm^2) Protein (>0.45g/L)**	**CT**	**Treatment**	**Outcome**
8	Paediatric	Female	Serum & CSF both positive	2 weeks	Headaches, behavioural changes, seizures	Normal	Abnormal consistent with encephalopathy	WCC: 43	No ovarian teratoma	Immunosuppression IVIG Rituximab	Recovered but with occasional aggressive outbursts
9	Paediatric	Male	Serum & CSF both positive. In the first episode, CSF was antibody negative at completion of treatment	Refer clinical	Historical (9 years prior) severe NMDAR encephalopathy Foot and spinal deformity, seizures, developmental delay ongoing	Not performed	Not performed	Normal	Not performed	No evidence of NMDAR relapse – Immunosuppression withheld Valproate increased for seizures Orthopaedic surgeries for foot and spinal deformities	Marked developmental delay Mobility improved with surgical interventions Seizures controlled
10	Paediatric	Male	Serum & CSF both positive	2 weeks :Transfer from out of NRA region	Ataxia, behavioural changes, loss of verbal skills, orofacial dyskinesia's	Not performed	Abnormal consistent with encephalopathy	WCC: 7	Not performed	Immunosuppression IVIG	On discharge improved mobility and behaviours but poor verbal skills
11	Paediatric	Male	Serum only tested and positive	1 month	Seizures, hallucinations	Normal	Mildly abnormal	Consent not given for LP	Not performed	Immunosuppression IVIG	Fully recovered

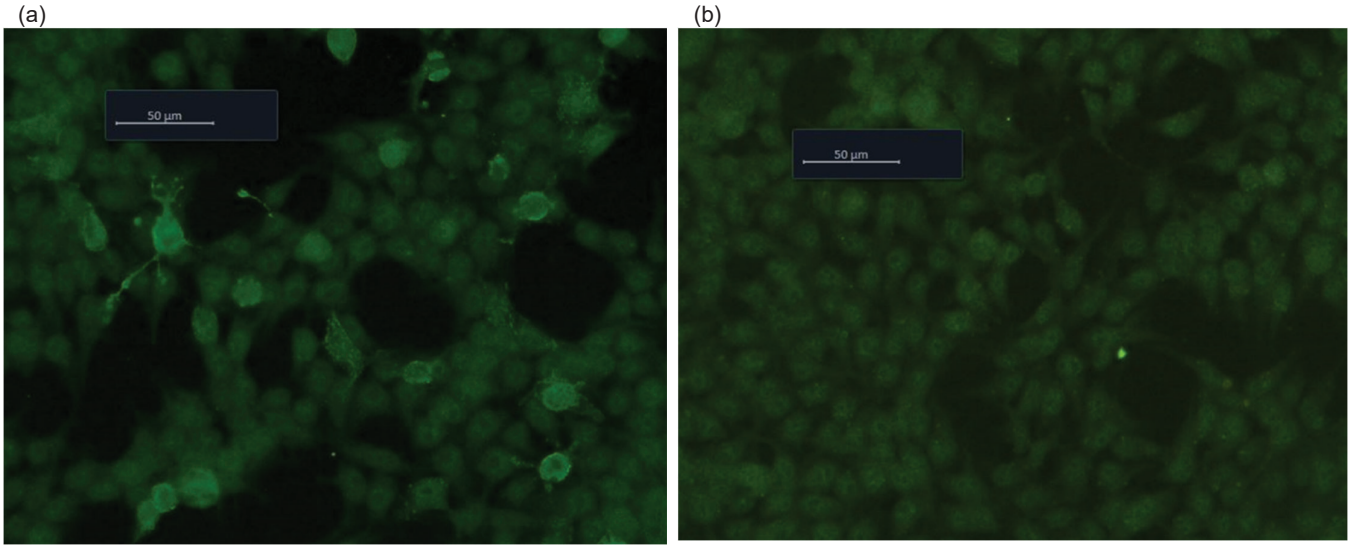

Figure 1. NMDAR antibody positive serum diluted 1:300, magnification x200 as expressed on Euroimmun (a) NMDAR transfected and (b) un-transfected EU90 cell line.

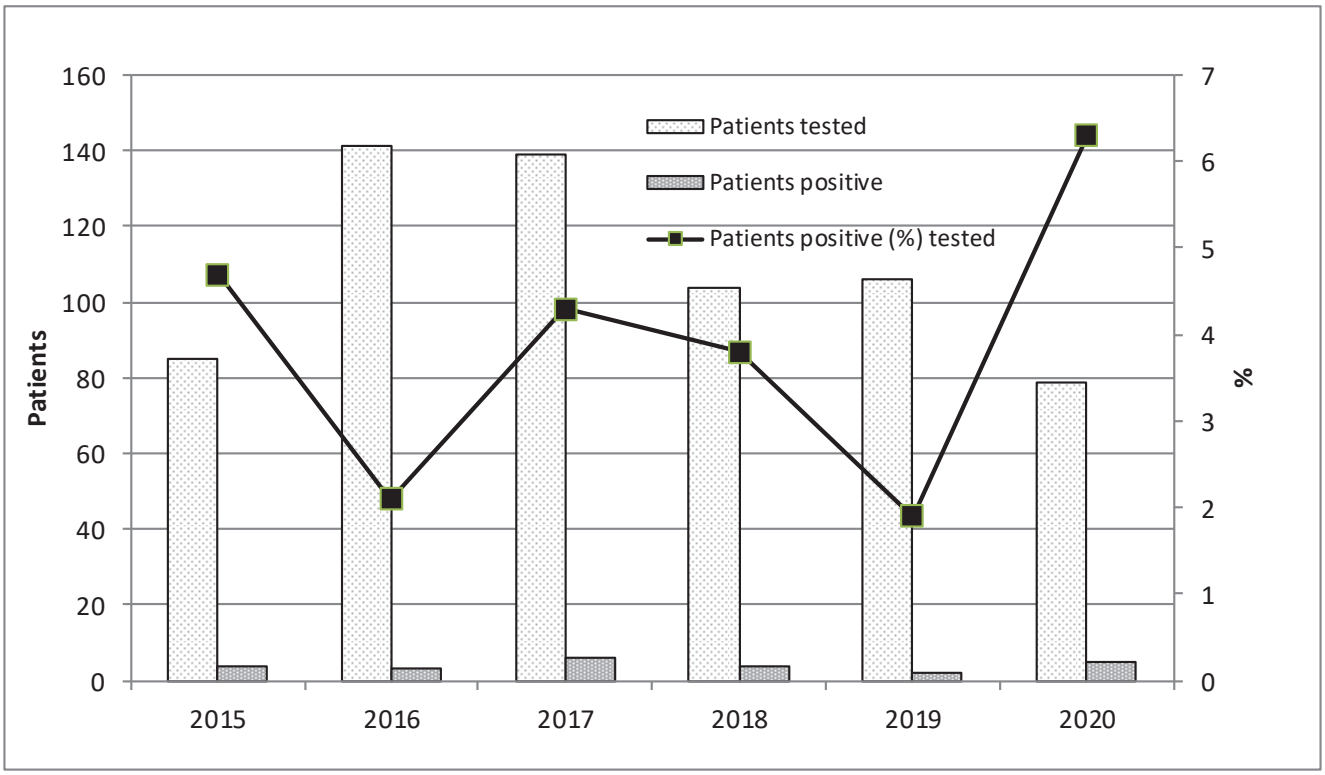

Figure 2. Frequency of NMDAR encephalitis cases identified at LabPLUS over a six year period (2015-2020) in a demand-managed neuroimmunology diagnostic service.

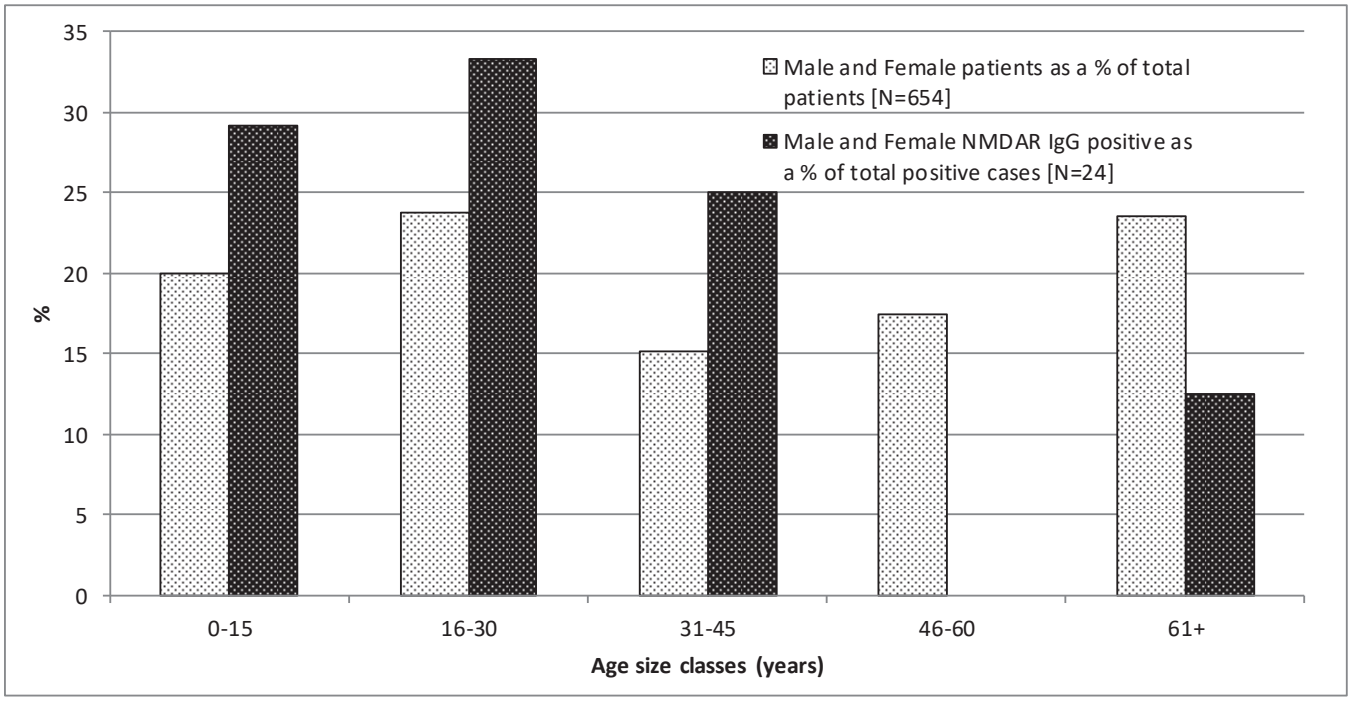

Figure 3. Age distribution of combined male and female patients tested with an added distribution of NMDAR IgG positive patients (combined male and female) represented as a proportion of total NMDAR IgG positive cases over the period 2015 – 2020.

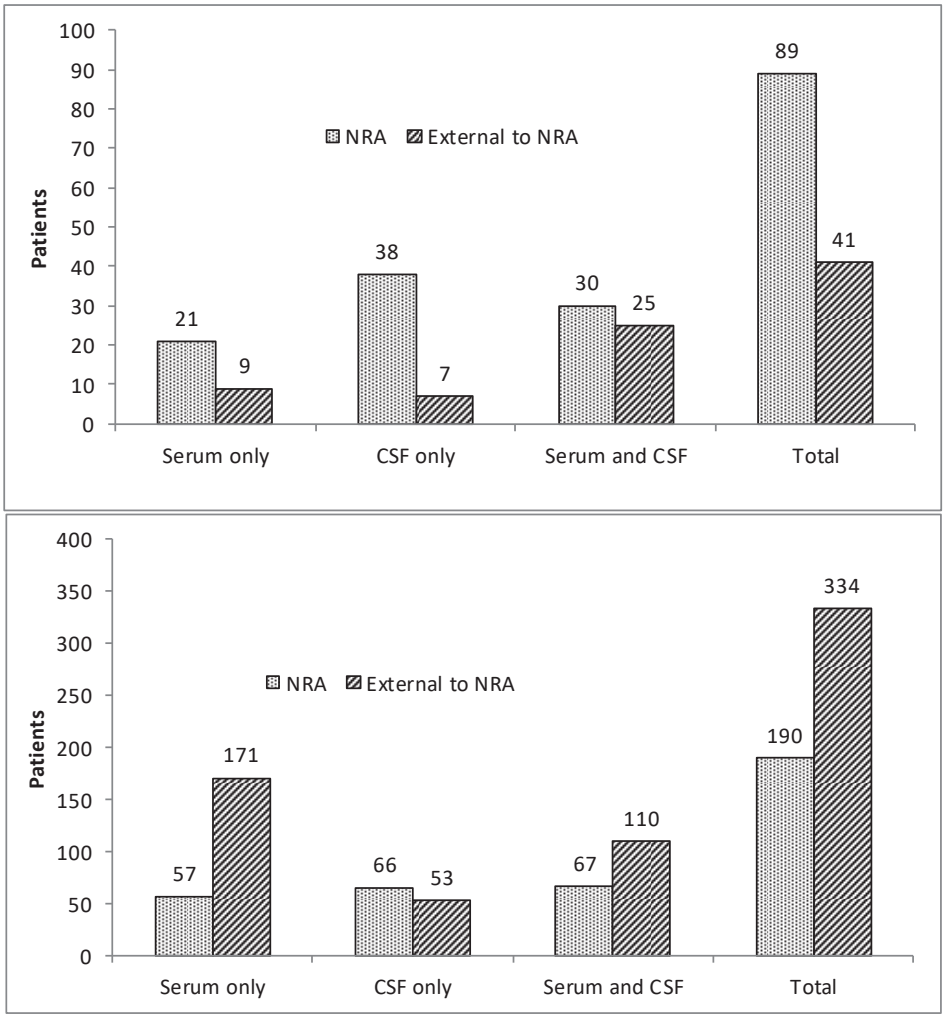

Figure 4. Distribution of referral site and specimen type for (a) paediatric and (b) adult patients tested for NMDAR IgG antibody at LabPLUS 2015 – 2020.

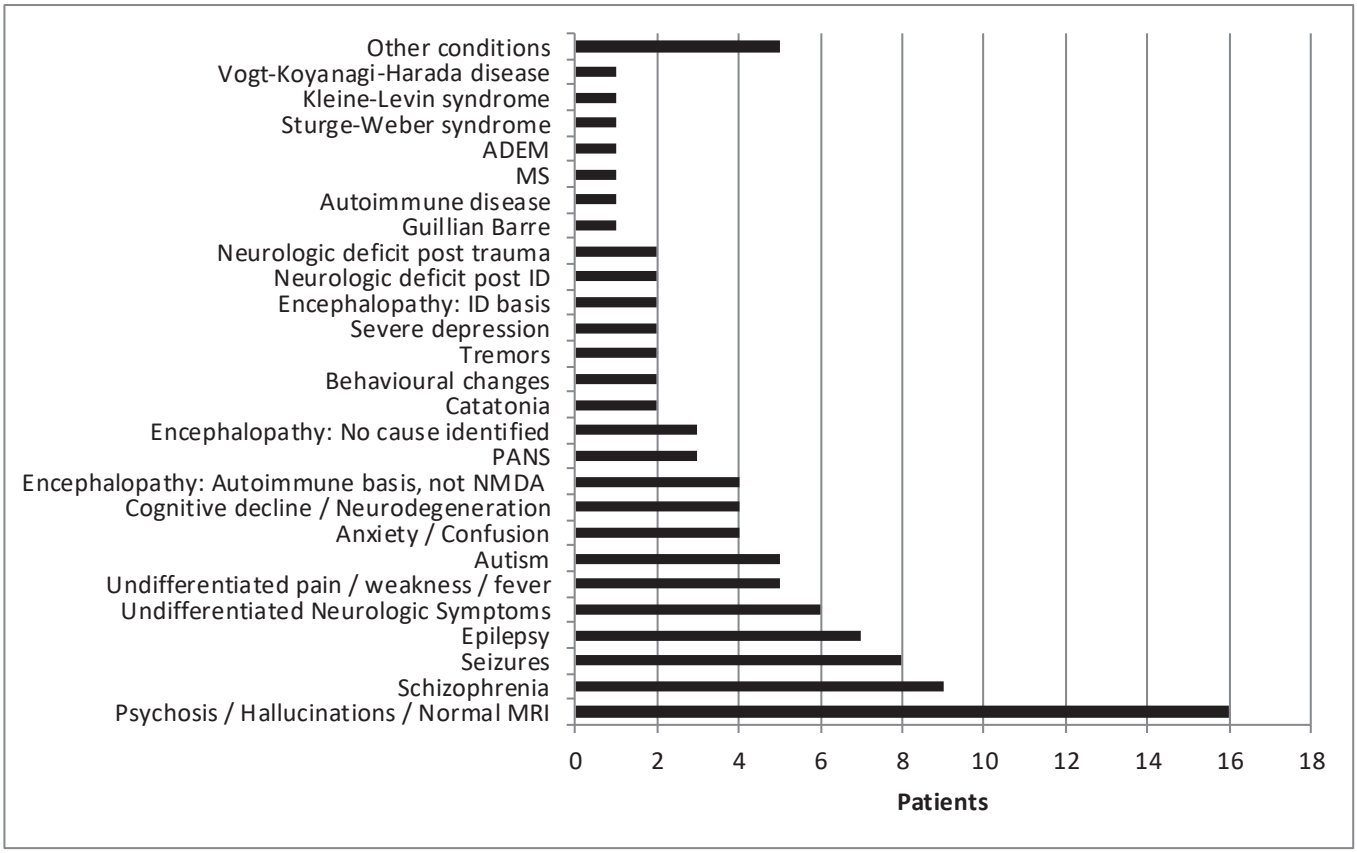

Figure 5. Confirmed diagnoses of 100 NMDAR antibody negative patients tested in the NRA over the period 2015 – 2020.

DISCUSSION AND CONCLUSION

Before discussing the sentinel findings from this retrospective analysis, it is also important to relate the body of work to the implementation of the first New Zealand diagnostic neuroimmunology testing service where NMDAR antibody testing was the flagship assay launched in 2015. The functional centrepiece of the service was to ensure neurologists acted as gatekeepers for all requesting.

The variety of symptoms from patients presenting with suspected autoimmune encephalitis, (AE) and in particular suspected NMDAR encephalitis, often pose significant challenges for those clinical teams that afflicted patients first encounter. An additional challenge for treating clinicians in non-neurological specialty is the regularly modified investigative algorithms that differ for adult and paediatric patients (10-11). The use of neurologists (adult and paediatric) in a gate keeping role was implemented to standardise the clinical approach to diagnoses. Tangible benefits that were expected to accrue were (a) that requests were appropriate and that the laboratory would not be overwhelmed with a non-specific volume of requesting; (b) that depending upon the investigation correct specimens were taken; (c) that assay TAT's were established on the basis of clinical need; (d) that communication lines with the pathology laboratory were robustly maintained and improved over time; (e) that neurologist involvement (diagnosis and treatment) was maximised for cases, the physicians having been at the forefront of the decision-making process; and (f) that the service was both fiscally viable and expandable. The expectation being that all of these stated benefits would then translate into improved patient outcomes.

From the original service planning meetings it was envisaged that if LabPLUS were able to deliver an appropriate service level for NMDAR antibody testing their neuroimmunology assay portfolio would expand over time. The hoped for service expansion was a rigorously planned exercise taking into account (a) the sequence of implementation of additional assays based upon clinical demand; (b) staff training requirements; (c) equipment procurement; (d) performance of technical validation studies with neurologist acceptance; and (e) IS resource availability. The authors fully understood that during the service expansion operational and performance standards for assays that had been implemented could not be allowed to be compromised.

The geographic site (within or external to the NRA) of referral of patients for testing was strongly associated with patient age. For adults, there was a ratio in excess of 2:1 for referrals outside the NRA region. For paediatric patient, there was still a 2:1 ratio but highest numbers were from within the NRA region.

As a direct consequence of the demand-managed service, in partnership with the neurologist gatekeepers, the laboratory was able to identify 24 cases of NMDAR encephalitis from 654 approved requests over the six year term giving an average frequency of disease in our tested population of 3.7% per annum. An article published in 2019 by Dalmau *et al.* stated that NMDAR encephalitis is a rare disorder with an incidence of 1-5 cases per million populations (12). Although this article is not intended to determine the frequency of NMDAR encephalitis in the New Zealand general population, the relatively high rate of antibody detection demonstrates the benefit of neurologist engagement ahead of the undertaking of the analytical procedure. Furthermore, the much higher rate (approximately 10 times) of antibody detection between requests for monospecific NMDAR (3.7%) versus requests for limbic encephalitis (0.4%) in roughly equivalent numbered patient cohorts is likely related to NMDAR encephalitis being the best characterised and most common form of limbic encephalitis (13). There is clear evidence from this study that in a demand managed service setting, with neurologist oversight, the provision of both mono-specific NMDAR as well as LE panel antibody testing is both appropriate and necessary.

The gender ratio of our tested population was 80/20 in favour of adults over paediatric patients and, somewhat surprisingly, there were similar numbers of males and females. For females tested numbers were consistent at approximately 20% of the total (N=380) per 15 year age bracket (years 0->61). By comparison the male tested population (N=274) demonstrated a bi-modal pattern with the initial peak being ages 0-30 years and then a secondary peak of ≥46 years age. Combined male and females (385/654; 59%) were aged ≤ 45 years of age, this age bracket accounting for 21/24 (88%) of NMDAR antibody positive cases. Seven of the twenty-four (30%) NMDAR antibody positive cases were from paediatric patients. Our data of a higher female incidence and prevalence in younger adults is broadly in line with published data (13) although our female prevalence was lower at a ratio of approximately 2:1, half of that which is generally quoted. This difference may be attributable to a relatively small antibody positive cohort (N=24). NMDAR encephalitis cases do occur in the older aged adults albeit at a very low frequency. In an observational cohort study by Titulaer *et al.* in 2013, 8/661 (1.2%) cases of NMDAR encephalitis were seen in patients aged ≥ 61 years (male N=4, range 62 -76 years; female N=4, range 62 – 85 years) (14). By comparison our cohort was entirely male at a much higher frequency of 12.5% of all antibody positive cases. Our higher frequency may be due to the fact that as patients age disease severity lessens (14). Patients with mild symptoms not seen by clinicians with specific expertise in autoimmune neurologic disease may not consider testing for NMDAR antibody potentially resulting in undiagnosed and/or mis-diagnosed cases.

From the NRA region 269 patients were reported as NMDAR antibody negative and overall 629/654 (96%) were reported as antibody negative. The obvious question to be posed is what is the exclusion value of a negative antibody result for NMDAR encephalitis? There are a number of published reviews and guidelines stressing the importance of testing CSF specimen type with or without a serum specimen as opposed to an independent serum specimen type due to higher comparative sensitivity of CSF during the acute stage of the disease (15-16). Of great concern was a published retrospective study by Guasp *et al*. in 2020 where, from 489 cases of confirmed NMDAR encephalitis, 75 (15%) patients returned a negative antibody test from an independent serum specimen. For those 75 patients the median time interval from symptom onset to antibody testing of 30 days was not statistically different to the 414 patients (35 day interval) who were seropositive on both CSF and serum specimens (16). The 75 seronegative patient subset were typically older and of male gender (16). This finding implies that use of an independent serum specimen will be challenged both by lack of sensitivity in the acute stage of the encephalopathy but also potentially in a subset of typically older males with the disease.

In our tested population, of the 23 patients who presented acutely with clinical features suggestive of NMDAR encephalitis, 20 had both a paired serum and CSF specimen taken. Six of the twenty patients (30%) had isolated reactivity in the CSF and as such, for these patients diagnosis and possibly treatment would have been delayed if only a serum specimen had been taken for antibody testing.

Clinical records were reviewed to determine the final diagnoses from 100/269 (37%) NRA region patients who were reported as antibody negative. The adult to paediatric split was 70/30 (patients) with twice as many female records reviewed compared with males. The proportions of adult/paediatric patients and female/male patients were in-line with the complete population. Overall, 75% of the 100 patient subset had a CSF specimen taken with or without serum (adult patients: 50/70, 71%; paediatric patients: 25/30, 83%). None of the 25 patients, where an independent serum specimen was originally taken, had any follow up specimens (CSF or serum) sent for analysis. Additionally, none of this group had a final diagnosis that was consistent with NMDAR encephalitis. On the basis of this subset patient review we believe that when NMDAR antibody results were reported as negative from our testing facility, particularly when the specimen type was CSF, that reported result had a very high likelihood for excluding NMDAR encephalitis as the pathology causing the patient symptoms.

Patients with NMDAR encephalitis will undergo a stage of psychosis which is variable from patient to patient in terms of severity, duration, and presentation within the constellation of symptoms associated with the disease (5,10). In our study we identified 5/10 (50%) acutely presenting confirmed NMDAR encephalitis patients from within the NRA region having psychoses as a component of their clinical presentation. Within the group of 100 NRA patients with negative NMDAR antibody results, 17 (17%; adult male N=4, adult female N=6, paediatric male N=2, paediatric female N=5) had psychotic symptoms. A CSF specimen was taken for 10 (59%) of the patients. None of the patients (a) had a final diagnosis of an encephalopathy; (b) had any follow up investigations; or (c) had any immunotherapy administered.

The now well recognised clinical stage of psychosis has led to an increased frequency of test requests for NMDAR antibody from psychiatrists in the setting of a patient presenting with a first episode psychosis. The 2016 Australian and New Zealand guidelines for investigations to be conducted for individuals presenting with first episode psychosis recommends testing for NMDAR and other antibodies (17). In the documented test listing and accompanying text there was an absence of commentary (a) regarding preferred specimen type; (b) the risks in the interpretation of results from independent serum specimens; and (c) regarding seeking advice from neurologists with expertise in autoimmune-based diseases. In two recently published articles the frequency of NMDAR antibody was determined by testing patients presenting with FEP (18,19). Across both studies the frequency of serum-based NMDAR antibody was 2.5% (11 antibody positive/441 patients). However, only four of the 11 antibody positive patients had either classical NMDAR encephalopathy symptoms (including the presence of ovarian teratoma) or were responsive to immunotherapy (18-19). Assuming all of the reactive reported antibody results were specific for the receptor, the implication is that serum antibody testing in such populations may give reactive results that are not associated with the well characterised encephalopathy.

With direct reference to the clinical and laboratory criteria for a definitive diagnosis of NMDAR encephalitis proposed by Graus *et al*. in their position paper in 2016 (10), all 23 patients that were NMDAR antibody positive on CSF and/or serum specimens met the case definition. It should be noted that the difference in the criteria for a diagnosis of AE versus NMDAR encephalitis is the (a) addition of MRI abnormality for AE; (b) the specific removal of reference to the presence of teratoma in AE; and (c) the replacement of reference of NMDAR antibody with detection of cell-surface, synaptic or onconeuronal antibodies for AE (10). Abnormal EEG or elevations in CSF white cell counts were specifically stated criteria for both AE and NMDAR encephalitis. For our 10 new diagnoses of NMDAR encephalitis seen in the NRA region, 4/5 (80%) patients had abnormal EEG findings, 6/9 (67%) patients had CSF pleocytosis, and 3/3 (100%) patients had an abnormal EEG and pleocytosis. Two out of four patients (50%) that only had a CSF investigation returned a normal white cell count. A single patient out of four (25%) that had both CSF and EEG investigations returned normal results for both. Interestingly, MRI investigations were performed on eight of the patients; a single patient (12%) returned an abnormal MRI. This finding endorses the recommendation from Graus and colleagues that abnormal MRI findings be restricted to the criteria for AE and not be included in the specific criteria for a case definition of NMDAR encephalitis.

Because of the clinical criticality for both diagnosis and initiation of treatment, our result TAT [defined as time from specimen receipt to time of issuing of the report] for any suspected case of AE is same day if the specimens are in the analytical area by 1000 hours. We were within this KPI for all 24 cases of NMDAR encephalitis and quicker if the patient was from within the NRA region. This consistently delivered speed

of service (which includes re-testing for new cases) allows physicians to either deliver or withhold immunotherapy with confidence. Apart from the electronic reporting facility, common in most if not all modern laboratories, for all antibody positive cases we additionally directly notify the requesting neurologist involved with the case immediately on completion of the testing. This last service feature ensures the rapid delivery of an appropriate treatment intervention irrespective of the clinical unit the patient is under admission to.

In summary, the findings from this retrospective study clearly demonstrate the benefit of having pre-analytical vetting of requests and familiarity of potential positive cases by neurologists. Within the framework of pre-analytical vetting, in conjunction with highly trained medical laboratory scientists supported by immunopathologists, we consistently deliver high value results within the clinically demanded time-frame for cases of suspected NMDAR encephalitis. The networking that has developed over time between the laboratory and the neurology teams throughout the country has ensured that for the majority of patients we now see from within the NRA region have CSF specimens taken for antibody testing. In the early years of the service, specimens referred from outside the NRA region were usually serum, however, due to the afore-mentioned networking efforts a higher proportion of CSF specimens are now being received. The high degree of specimen (CSF) compliance, speed of service and application of pre-test probabilities by neurologists all come together to maximise the benefit to the patients under investigation. Feedback from both adult and paediatric neurologists for the service that has been developed and delivered is uniformly positive and collegial. The success story of the mono-specific NMDAR antibody testing allowed the further development of the neuroimmunology diagnostic service that is now offered at LabPLUS.

ACKNOWLEDGEMENTS

The authors would like to acknowledge and thank Heather Deehan (Quality and Information Technology Support Officer, Virology and Immunology Department) for providing the data extract which enabled this article to be written.

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