Comparison of antibody assays in anti-NMDAR encephalitis

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Background

Encephalitis associated with antibodies directed against neuronal surface targets is an expanding field in neurology, and the most common entity in this group is the anti-N-Methyl-D-Aspartate receptor (NMDAR) antibody encephalitis [1] This usually presents as a combination of encephalopathy, psychiatric symptoms, seizures, autonomic disturbances and movement disorders.[2] Antibody detection is a key step for diagnosis, but common techniques relying on linearized antigens such as ELISA are not adequate. Current "conformational" techniques for identification of NMDAR-antibodies are: a) immunohistochemistry (IHC) on rat brain slices, where antibodies binding on the neuronal surface is recognized through the identification of a specific hippocampal staining pattern;[3] b) a cell-based assay (CBA), where antibodies bind to the target antigen expressed on the surface of an engineered cell, that can be either live (L-CBA) or fixed (F-CBA);[4] c) a commercial CBA that uses fixed cells (C-CBA), (*Euroimmun, Lubeck*) that is currently used by most laboratories. Although some evidence suggests that F-CBA is superior to L-CBA, and that IHC is the most sensitive method, systematic studies comparing the different assays are lacking.[5]

Methods

We retrospectively collected samples sent to the Oxford Neuroimmunology service for possible autoimmune encephalitis that were originally tested with our in house L-CBA. We intentionally selected some patients with positive results and doubtful diagnoses. All samples were tested on L-CBA, F-CBA and IHC, and a subgroup was tested on C-CBA. The binding was scored according to a semi-quantitative scale (figure 1). After testing, the diagnosis of NMDAR-Ab encephalitis was classified as definite, possible or unlikely based on the judgement of the referring physician (figure 2).

Figure 1: example of results in different assays

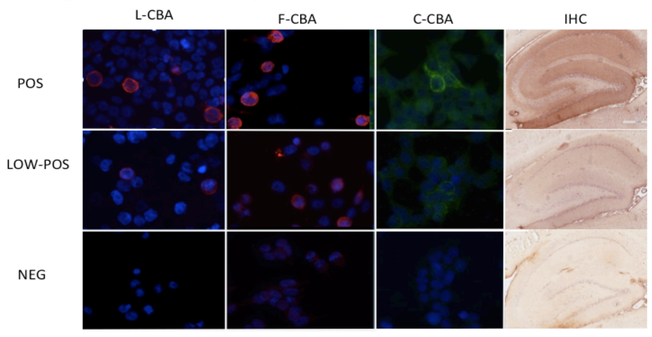
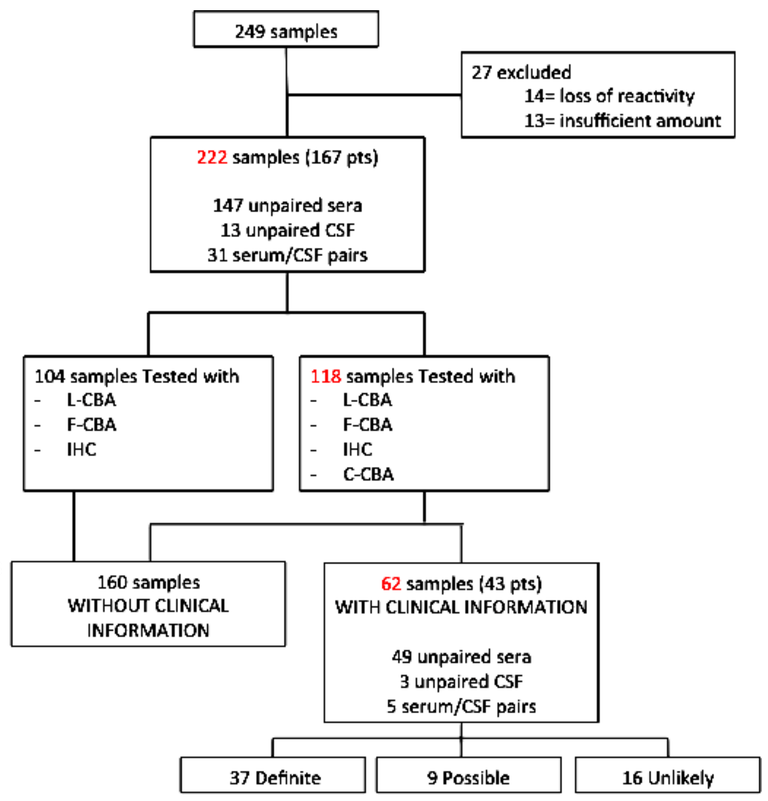


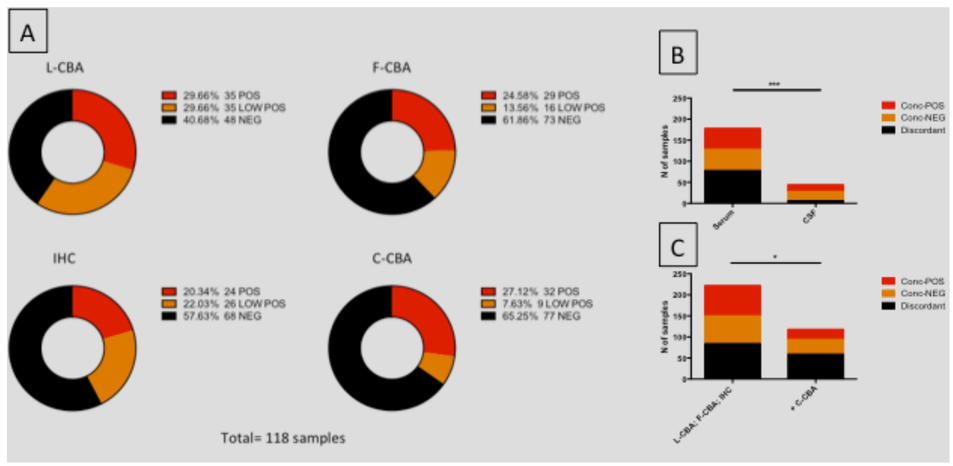
Figure 2: study algorithm



Results (1)

A total number of 222 samples were collected and tested (figure 1). Results are reported in Figure 3
concordance between the results in 3 assays was found in 138/222 samples (62 %), but was more frequent with CSF samples (38/44) compared to sera (100/178)(figure 3, A); in the subgroup of samples tested also with C-CBA (n=118), concordance of results between 4 assays was present in a consistently lower percentage (figure 3, B)

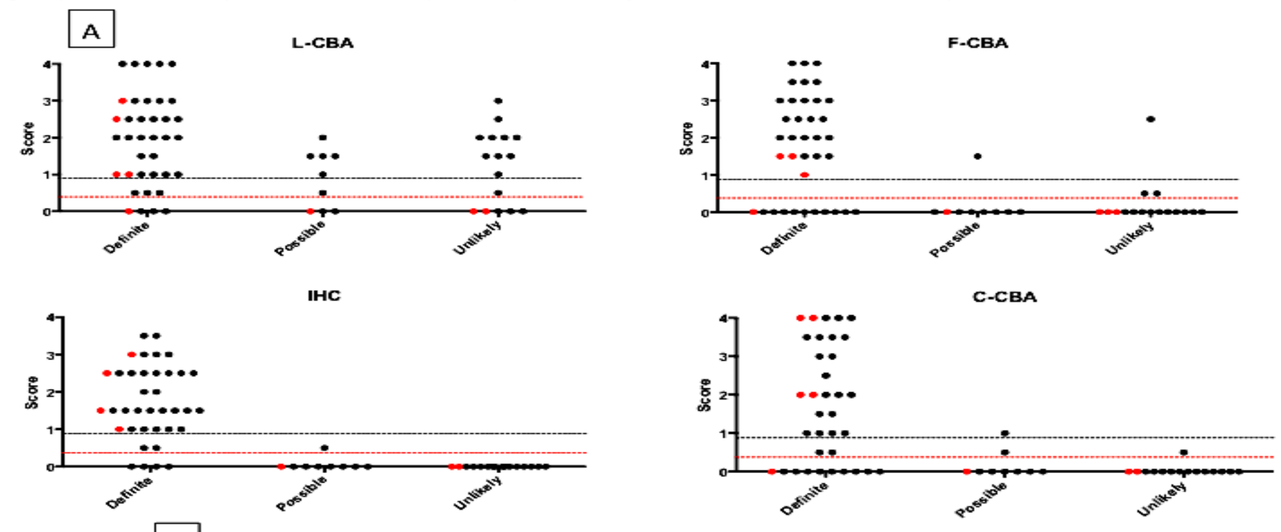
Figure 3: results summary. A) Positive and negative samples in different assays; proportion of discordant samples compared between 3 assay and 4 assay group (B) and serum and CSF (C)



Results (2)

Clinical information were available for 43 patients (59 samples) that were classified as definite (21/43), possible (8/43) and unlikely (14/43). Overall, the results in 35 definite samples were 29/35 (82.9%, L-CBA), 25/35(71.4%, F-CBA), 30/35 (85.7%, IHC) and 23/35 (65.7%, C-CBA). (figure 4). As expected from the biased selection, 14/25 unlikely/possible samples were positive with L-CBA compared to 2/25 with F-CBA, 1/25 with C-CBA and 0/25 with IHC. Only 5 serum/CSF pairs with clinical information were available (figure)

Figure 4: results in samples with clinical information. A) Results in different diagnostic groups; red dots represent CSF samples. B) Summary of results in 5 serum/CSF pairs.



B

		L-CBA		F-CBA		IHC		C-CBA	
		Serum	CSF	Serum	CSF	Serum	CSF	Serum	CSF
1	Definite	-	+	-	+	+	+	+	+
2	Definite	+	+	+	+	+	+	+	+
3	Definite	+	+	+	+	+	+	+	+
4	Possible	-	-	-	-	-	-	-	-
5	Unlikely	+	-	-	-	-	-	-	-

Results (3)

Endpoint titrations with L-CBA, F-CBA and IHC were obtained for 14 samples (3 CSF). In 10/13 IHC showed the highest endpoint titration and in 6/14 F-CBA showed the lowest (Friedmann test, p=0.0001)

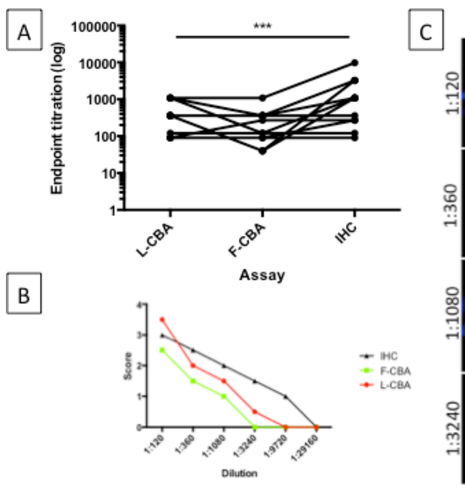


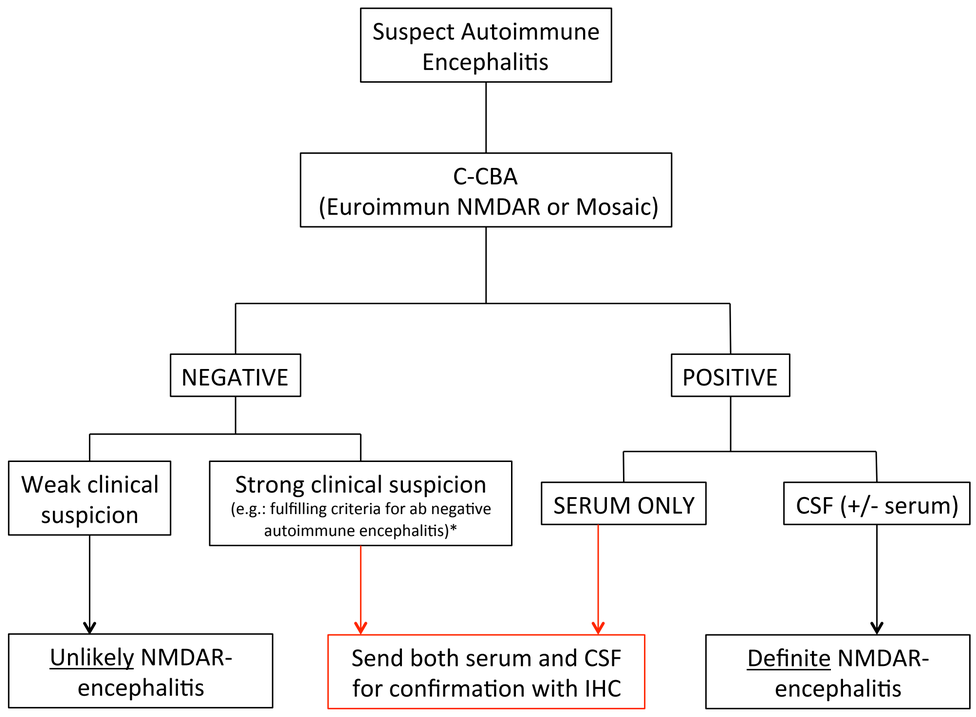
Figure 5: Endpoint titrations A) Summary of endpoint titrations in 14 samples. B and C) IHC shows the highest endpoint titration in an illustrative sample

Conclusions

Our data showed poor concordance between assays. IHC showed the best performance, identifying the highest number of positive samples in the definite group (85.7%) and none in the unlikely group, and the F-CBA was not very sensitive (71.4%). The L-CBA was also sensitive (82.8%), but considering the positive results in patients with unlikely diagnosis (biased selection), showed poor specificity, and interpretation of results requires caution. Interestingly, the C-CBA, despite high specificity, showed poor sensitivity in our hands (34.3%) which is of concern as this is the only assay available worldwide. Insufficient CSFs with clinical information were available to make a meaningful comparison.
In summary, as reported (5),to identify NMDAR antibodies, IHC performed with CSF should be the assay of choice when possible, and the target antigen can be confirmed with a sensitive CBA. Negative results with C-CBA do not exclude the diagnosis, and further testing with either L-CBA or IHC should be performed in patients where clinical suspicion is strong.
A recently published consensus for the definition of autoimmune encephalitis suggests, in patients fulfilling clinical criteria and negative at frist line screening, to test the sample in a specialized laboratory able to perform specific techniques such as IHC.(6) Supported by our data, we propose a diagnostic algorithm that could improve the diagnosis of anti-NMDAR encephalitis.

We thank EUROIMMUN AG for providing the slides

Figure 6: Diagnostic algorithm for anti-NMDAR encephalitis
Patients with suspect autoimmune encephalitis will be tested with C-CBA in the referral neuroimmunology laboratory. In consideration of the good specificity of the assay, positive results with concordant clinical phenotype can be considered definite NMDAR encephalitis. Patients with either serum positivity only or negative with a suggestive clinical phenotype should have their samples sent for testing in a specialized laboratory able to perform IHC.



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