Prevalence and clinical characteristics of serum neuronal cell surface antibodies in first episode psychosis: a case-control comparison study

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Summary

Background

Psychosis is a common presenting feature in antibody-mediated encephalitis, where prompt recognition and treatment usually leads to remission. It is not known whether people with circumscribed schizophrenia-like illnesses also have these antibodies, especially antibodies against the NMDA receptor (NMDAR), more commonly than do healthy controls.

Methods

We recruited patients with a first episode of psychosis and less than 6 weeks of treatment with antipsychotic medication from mental health services. We completed standardised symptoms rating scales (PANSS, ACE-III, GAF) at baseline, and tested serum for antibodies against NMDAR and four other neuronal cell surface targets: LGI1, CASPR2, $GABA_A$ receptor ($GABA_AR$) and AMPA receptor (AMPAR) using live cell based assays. Treating clinicians assessed outcomes at 6 months. We recruited healthy controls with similar characteristics in age, gender and ethnic origin as the cases.

Findings

20 (8.8%) of 228 first-episode psychosis patients had serum antibodies against one or more of the neuronal cell surface antibodies, compared with 4 (3.8%) of 105 controls which was not significant ((odds ratio (OR) 2·4 (95%CI 0·8 - 7·3) unadjusted). These associations remained non-significant when adjusted for current cigarette smoking, alcohol consumption and illicit drug use. 7 (3·1%) of 228 first-episode psychosis patients had NMDAR antibodies, compared with 0/105 controls. The other individual antibodies were not different between groups. Antibody-positive patients had lower PANSS positive and PANSS total scores, and catatonia scores, than patients without antibodies. Patients had comparable scores on other PANSS items, ACE-III and GAF at baseline, and there was no difference in outcomes.

Interpretation

A proportion of patients with first episode psychosis have antibodies against NMDARs that might be relevant to their illness. These patients do not differ from those without NMDAR antibodies in clinical characteristics. Therefore our study suggests that the only way to detect patients with these potentially pathogenic antibodies is to screen all those with first-episode psychosis at first presentation.

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Research in context

Evidence before this study

We searched MEDLINE up to August 2016 for all studies on the prevalence of neuronal cell surface antibodies in patients with psychosis. We used search terms "antibod*" AND "psychosis"OR"schiz*" AND "NMDA*"OR"N-methyl-D-aspartate receptor" OR "LGI1" OR "CASPR2" OR "mGluR5" OR "AMPA*" OR "GABAA*" OR "GABAB". We excluded non-English language articles. Several studies have examined the prevalence of serum NMDA receptor antibodies, with one systematic review and meta-analysis of 9 studies, including 3387 participants showing a three times greater (odds ratio 3·1) NMDA receptor antibody positivity in patients with schizophrenia or schizoaffective disorder, bipolar affective disorder or major

depressive disorder compared with controls. Subsequent studies have reported varying results. One study in children found 11.6% of 43 NMDA receptor IgG antibodies in those with psychosis, and none in 43 healthy or disease controls. The largest studies failed to find any seropositive psychosis cases or found equivalent prevalence in healthy controls. One study found similar seropositivity for LGI1 and CASPR2 antibodies in patients with established schizophrenia and healthy controls. Another found no antibodies against AMPA receptor in 459 participants with psychiatric illness or healthy controls. No studies have examined the prevalence of $GABA_A$ receptor antibodies in patients with psychosis.

Added value of this study

We found no overall differences in prevalence of antibodies against neuronal cell surface proteins in the serum of well-characterised patients with first episode psychosis and with less than 6 weeks medication compared with a matched healthy control sample. NMDAR antibodies were present in the serum of patients with first-episode psychosis whereas none were detected in controls. This provides a better estimate of the prevalence of neuronal cell surface antibodies associated with the onset of psychotic illness.

Implications of all the available evidence

Our finding that NMDA receptor antibodies can be detected in the serum of patients with a first episode psychosis at a higher rate than controls supports existing evidence

that reduced activity of the NMDA receptor has an important role in schizophrenia. Antibodies to the NMDAR at the onset of illness may be the basis for these findings in some patients. Patients with these antibodies might respond to treatment with immunotherapy and screening of patients with first-episode psychosis is therefore suggested.

Introduction

Autoantibodies to neuronal cell surface receptors and related proteins have been described in association with encephalitic syndromes that frequently include psychiatric symptoms, usually psychosis, as a prominent part of the phenotype. Anti-N-methyl-d-aspartate receptor (NMDAR) encephalitis, first described in 2007,[1] is the most common of these. It is a multistage encephalitis caused by antibodies to the GluN1(NR1) subunit of the NMDAR which presents initially with psychiatric symptoms in over two thirds of patients, before recognition of neurological symptoms which include cognitive deficits, seizures, autonomic instability and movement disorder . [2]

Several autoimmune encephalitides have subsequently been identified associated with autoantibodies to other cell surface antigens , including LGI1,[3] CASPR2,[3] AMPAR[4] and $GABA_AR$.[5] Psychiatric or behavioural manifestations are commonly described,

and there are occasional case reports of patients with purely psychiatric presentations with these antibodies. [5,6]

In autoimmune encephalitis associated with VGKC-complex antibodies, the antibodies are usually directed against the neuronal surface antigens LGI1 and CASPR2, which are components of the VGKC complex: these antibodies are thought to be pathogenic. [3] There are further cases when the VGKC-complex antibodies do not bind LGI1 or CASPR2.[7,8] It is likely that some bind intracellular, non-pathogenic, epitopes on the VGKC-complex, and are therefore not clinically relevant, [7] although one study in children suggests that they may still be a marker for immune responsive neuroinflammatory conditions.[8]

In general, for these encephalitides, early treatment with first line (e.g. steroids, plasmapheresis and/or intravenous immunoglobulins) or second-line (e.g. rituximab or cyclophosphamide) immunotherapy is associated with good outcome and many patients are able to return to a premorbid status. [9,10]

Following initial recognition that these syndromes can cause psychosis as a prelude to a wider encephalitic picture, there has been considerable interest as to whether these autoantibodies are associated with psychotic symptoms without the emergence of other features of encephalitis, in patients presenting to psychiatric services.

In 2010 we identified serum antibodies to the NMDAR or VGKC-complex in 3 out of 43 (6·3% (95% CI 1·9-16·5%) of patients presenting to a first episode psychosis service.[11] A systematic review and meta-analysis showed an odds ratio (OR) of 3·1 for serum NMDAR antibody positivity in patients with schizophrenia or schizoaffective disorder, bipolar affective disorder or major depressive disorder compared with controls,[12] and a recent study of serum NMDAR antibodies in children has confirmed this finding.[32] However, there is considerable heterogeneity in terms of diagnosis, duration of illness, and the assay method used for detection of antibodies in these studies, and some large case-control studies have found no difference between patients with psychosis and controls in rates of NMDAR,[13,14,15] or LGI1/CASPR2[15] antibodies in serum.

In this study we aimed to provide a better estimate of the prevalence of NMDAR and other disease-relevant neuronal cell surface antibodies in the serum of patients with a first episode psychosis, compared to a group of healthy controls with similar age, gender and ethnicity characteristics. Given the association between autoantibody-mediated CNS disease and cognitive impairment [16,17] and to investigate further the possibility that autoantibody positivity might delineate a distinct phenotypic subgroup of patients with psychosis, we also aimed to characterise the clinical and cognitive profile of our subjects. We measured antibodies in serum rather than CSF both to align with clinical practice in the UK, and because of the experience in encephalitis,

where antibodies are detected at a higher rate in serum than CSF, especially early in the course of illness.[2]

While there is evidence that non-IgG antibodies (ie IgM, IgA) may have pathogenic potential within the CNS,[18] these are very uncommonly detected with live cell assays (AV, LJ unpublished data) and the study was restricted to IgG antibodies only.

Methods

Study design and participants

35 sites across England recruited patients experiencing a first episode of psychosis who were in contact with early intervention, community, or inpatient mental health services. Inclusion criteria comprised age between 14 and 35 years, less than 6 weeks on antipsychotic medication, and a score of 4 or more on at least one of the following Positive and Negative Syndrome Scale (PANSS)[19] positive items: 1 (Delusions), 3 (Hallucinations), 5 (Grandiosity), 6 (Suspiciousness), or PANSS General item 9 (Unusual Thought Content). Exclusion criteria included: suspected drug induced psychosis or the presence of neurological disorder (eg head injury, multiple sclerosis). The Local Research Ethics Committee approved the patient study, reference 12/EE/0307.

Control participants were recruited from the general population in Cambridge, UK as part of a separate study (Local Research Ethics Committee reference 08/H0308/5). Inclusion criteria included age over 16 years, with no personal or family history of mental illness. The control sample was similar in age, gender and ethnicity to a typical sample of first episode psychosis patients, and was similar to the current sample on these demographics (Table 1).

Procedures

Written informed consent was obtained from every participant. Patients were assessed at a single baseline assessment session with the following clinical measures: Psychotic symptoms: Positive and Negative Syndrome Scale (PANSS)[19] with positive, negative and general psychopathology symptoms sub-scores; general level of functioning for people with psychiatric disorders: Global Assessment of Functioning (GAF),[20] catatonic symptoms: Bush-Francis Catatonia Rating Scale (CRS), [21] brief cognitive assessment: Addenbrookes Cognitive Exam-III (ACE-III). [22]

PANSS rating concordance was assessed: Research assistants involved in data collection rated a standardised video of a PANSS interview. Inter-rater reliability was good: each RA score was within 1 S.D of the mean. 6 month outcomes were assessed from notes by treating clinicians, including GAF and illness course.

All patients and controls gave a venous blood sample; patient samples were taken at initial clinical assessment.

Antibody assays

Autoantibody testing was undertaken in Oxford using assays in routine clinical use. [2,23] A live cell-based assay was used for the detection of IgG antibodies to NR1 subunit of NMDAR, the VGKC-complex associated proteins LGI1 and CASPR2, α1 and γ2 subunits of $GABA_AR$ and AMPAR, as described previously. [3,5,23] Binding to the cell membrane was scored by fluorescence microscopy, with a visual score ranging from 0-4.[2] The titre of each antibody was given as the dilution of serum providing a score of 1. All assays were repeated, checked for IgG specificity, as for routine diagnosis, and scored separately and blind to diagnosis, on each occasion.

VGKC-complex antibodies were measured using a radio-immunoprecipitation assay of VGKC complex proteins labelled with 125I-a-dendrotoxin and precipitated with patient sera. [23]

Anti-nuclear antibodies (ANA), as an additional test of autoimmunity, were measured by direct immunofluorescence.

Statistical Analyses

We analysed data using SPSS version 22. We used t-tests to compare age and χ 2 tests to compare categorical demographic and lifestyle variables between first-episode psychosis patients and controls and the prevalence of neuronal cell surface antibodies. We used Odds Ratios (unadjusted, and adjusted for smoking, alcohol and illicit drug use) to compare the prevalence of antibodies between first-episode psychosis patients and controls. Likelihood ratios (unadjusted) were used to compare the prevalence of NMDAR and LGI1, as odds ratios could not be calculated. because of null values in the control group.

We used χ^2 tests and t-tests (or Mann-Whitney U tests with non-normally distributed data) to test for associations between clinical and cognitive test variables and antibody status in patients with first episode psychosis. For all analyses significance level was set at p=0.05.

Results

The 228 first episode patients and 105 healthy controls were similar in age, gender and ethnic origin (table 1). The youngest first-episode psychosis patient was aged 16 years. Patients were more likely to be current cigarette smokers than controls (n=120 (53%) of patients and n=16 (15%) of controls ($p<0.001$)) and more likely to be current users of illicit drugs n=57(25%) patients compared with n=14(13%) controls

p=0.015, whereas control subjects were more likely to be current alcohol drinkers n=82 (78%) controls compared with n=105 (46%) patients (p<0.001%)

On antibody testing 20 (8.8%) of first-episode psychosis patients were positive for any one neuronal cell surface antibody compared to 4 (3.8%) controls (table 2), but this difference was not significant. Serum NMDAR antibodies were more prevalent in patients (n=7; 3.1%) than in controls (0, 0%, p=0·02). VGKC-complex antibodies were present in 11 (4.8%) patients and 3 (2.9%) controls (p=0.38). One patient was positive for both NMDAR and VGKC-complex antibodies. The first-episode psychosis patients and controls did not differ in the presence of serum antibodies against LGI1, CASPR2, and $GABA_AR$ antibodies (table 2). No patients or controls had AMPAR antibodies. Overall, titres of those with positive antibodies were low (table 2). None of those with VGKC-complex antibodies had antibodies against LGI1 or CASPR2.

As the patient and control groups differed in rates of alcohol use, cigarette smoking and illicit drug use, adjusted odds ratios were calculated, group differences remained non-significant.

None of the patients with neuronal antibodies had ANA antibodies, and there was no significant difference in ANA antibodies between controls 9 (8.6%) and patients (7 (3.1%), OR = 0·50 (0·2-1·4).

Clinical and cognitive phenotype

The neuronal cell surface antibody positive and negative first-episode psychosis groups were compared on assessments of clinical and cognitive symptoms (table 3). Both groups had levels of psychotic symptoms that indicate a moderate level of illness (PANSS total 69.0-77·6), with antibody-positive patients having lower mean PANSS Positive scores (19·1 (sd 3·7)) than antibody-negative patients (21·8 (sd 6·1) $p<0.01$) and lower mean PANSS total scores (69·0 (sd 17·2)) in antibody positive patients than antibody negative patients (77·6 (sd 17·8); $p=0.05$). Both groups had low levels of catatonia symptoms, with antibody-positive patients having lower total scores (mean 0·60 (sd 1·1) than antibody negative patients (mean 2·2 (sd 3·7), $p<0.001$).

Both groups also had impairment in their cognitive functioning (mean ACE-III 81·8) and were moderately functionally impaired with mean GAF scores of around 50, but with no significant differences in these parameters between antibody-positive and antibody-negative groups (table 3).

Outcome data

Patients positive for autoantibodies were followed up 6 months after baseline assessment, to investigate clinical course and outcome. There was no significant difference in the number of increased contacts with mental health services (either hospital admission or treatment with a home treatment/crisis service) between the

antibody-positive patients (mean 0.4 (sd 0.6) and the antibody-negative patients (0.5(sd 0.7) p= 0.51) nor on the mean follow-up GAF ratings (antibody positive 66.5(sd 15.5), antibody negative 63.4 (sd 15.7) p=0.52). There were no cases of encephalitis in the patients, or development of neurological symptoms, as assessed by their treating psychiatrist

Discussion

In the largest study so far examining the prevalence of neuronal cell surface antibodies in serum of patients with first-episode psychosis, we have shown that . NMDAR antibodies are more prevalent in patients with first-episode psychosis than in the healthy control group. The prevalence of antibodies against $GABA_AR$, LGI1 and VGKC-complex did not differ between the groups. Odds ratios adjusted for current smoking, alcohol consumption and illicit drug use were also non-significant. There were no major differences in clinical phenotype between antibody- positive and antibody-negative cases, however patients who tested positive for neuronal cell surface antibodies scored significantly lower on PANSS positive items and PANSS total score, as well as the Catatonia rating scale.

Our NMDAR antibody findings corroborate an earlier report[11] and provide support for the many converging lines of research evidence suggesting that NMDAR hypofunction plays an important part in schizophrenia. [24] Susceptibility genes for

schizophrenia, both common and rare variants, are particularly associated with glutamatergic transmission and with the adaptive immune system.[25] Pharmacological blockade of NMDAR produces the full spectrum of symptoms seen in schizophrenia, as well as the neuropathological findings of reduced numbers of inhibitory GABAergic interneurons and reduced dendritic spine density that are compatible with a model of NMDAR blockade.[24,26] Antibodies to the NMDAR at the onset of illness may be the basis for these findings in some patients.

For all antibodies except VGKC-complex and ANA, a live cell-based assay was used to assess antibody status. In a live cell-based assay the serum to be tested for auto-reactivity is incubated with live HEK293 cells, previously transfected with plasmids encoding the specific antigen subunit(s), before these cells are fixed.[2] By contrast, both commercial assays and the assays performed by most other laboratories involve fixation and/or permeabilisation of cells before incubation with serum or CSF.[27] It is not known what effects fixation and permeabilisation (and other inter-assay differences) may have on the relative sensitivities and specificities of these assays. This is particularly true when the assay is used in psychiatric populations where, unlike in some autoimmune CNS disorders, eg neuromyelitis optica,[28] there is no well-established and clinically-defined group against which such sensitivity and specificity can be assessed independently of autoantibody status. It is likely, however, that in assays other than live cell-based assays, permeabilisation of the cell membrane

exposes intracellular antigens and so seropositivity might indicate the presence of antibodies that can bind intracellular epitopes; these antibodies would not be expected to be pathogenic.

VGKC-complex antibody levels, as measured by radioimmunoassay, did not differ significantly between first-episode psychosis patients and controls, and none of those with VGKC-complex antibodies had antibodies against LGI1 or CASPR2. The relevance of these antibodies in psychosis is, therefore, uncertain, and a high level of VGKC-complex antibodies, by themselves, might not be clinically relevant in psychosis.

In this study, antibody-positive patients had a lower level of catatonic and psychotic symptoms. The absolute difference in symptom scores was modest, and does not reflect clinically meaningful differences. However, it does contrast with a previous case series of patients with NMDAR antibodies and psychosis where patients were described as being more psychiatrically unwell, with more catatonia, cognitive impairment, and with adverse reactions to antipsychotics.[29] These previous cases might have been subject to selection bias, whereby clinicians preferentially requested the antibody test only if the patient presented with atypical features suggestive of encephalitis.

The prevalence of IgG antibodies detected in this study is higher than that described in most other groups of patients with either first-episode psychosis or longstanding illness (ranging from 0 to 1.6%[13,14,15]). A notable exception is a study in children with psychosis, tested a median of 5 weeks after onset of symptoms, which found 11.6% NMDAR IgG antibodies, with none in healthy or illness controls[34]. A possible explanation is the short length of treatment with antipsychotics in our group Antipsychotics have been shown to have immunomodulatory properties,[30] even showing therapeutic promise in an animal model of autoimmune encephalitis.[31] Some studies have detected IgM and IgA antibodies to neuronal targets, [14,15,32] but we did not specifically look for IgM or IgA antibodies in this study as previous attempts to demonstrate them on live NMDAR assays were unsuccessful (AV, LJ unpublished data).

A limitation of the study is that we did not collect CSF, owing to the impracticality of doing lumbar punctures in routine UK mental health settings. Detecting antibodies in CSF provides a more definitive indicator that they are having a central nervous system effect in that individual. However a recent study has shown that antibodies that have crossed the blood brain barrier tend to bind to the brain, with the brain acting as an 'immunoprecipitator', and will therefore not be measurable in CSF.[33] An absence of antibodies in CSF does not, therefore, prove that antibodies are not causing illness in that individual. A further limitation is that antibody status was measured at

a single time-point only. We were therefore unable to establish whether, as is the case in many autoimmune diseases, autoreactivity precedes the development of symptoms. If this is the case, it is plausible that a 'second hit' causing disruption of the blood-brain barrier would be required for pathogenic antibodies to access the CNS and affect neuronal function, as has been suggested elsewhere.[15] We looked only at patients with first-episode psychosis. These antibodies might be associated with other acute or subacute onset neuropsychiatric disorders with overlapping features with encephalitis, such as depression, dementia, or acute confusional states.

Further work is now required to establish the specificity and pathogenicity of these antibodies in the context of psychosis. The demonstration that even a small proportion of cases of schizophrenia-like psychosis have an autoimmune basis would have important ramifications for nosology and for treatment, since they might respond to immunotherapy rather than antipsychotics or psychological interventions. Our study suggests that, at present, the only way to detect patients with these potentially pathogenic antibodies is to screen all those with first-episode psychosis at first presentation.

Author contributions:

BRL, PBJ, PH, AV contributed to the initial study design

JH, EP-C, PPiP study group contributed to patient data collection

LS undertook the control sample collection

LJ, BL, HF, BF, AV were responsible for the antibody assays and sample analysis

BRL, EP-C, TP, JH, JM, HG, contributed to data analysis

All authors have contributed to the manuscript and have give final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declarations of interest

BRL, EP-C, TP, PBJ, LS, HF, BF report no conflicts of interest. AV and the University of Oxford holds patents and AV receives a proportion of royalties from Athena Diagnostics and Euroimmun AG.

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