**When should we test for voltage-gated potassium channel complex antibodies? A retrospective case control study**

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**Abstract**

Patients with voltage-gated potassium channel (VGKC)-complex antibodies are increasingly recognised, having central, peripheral or combined phenotypes. With increasing awareness, more patients are tested and the clinical spectrum expanding. Consequently, clinicians may be uncertain as to which patients should or should not be tested. Previous studies have identified common clinical features, but none has looked at the usefulness of these in predicting seropositive disease.

We conducted a case-control study of patients tested for VGKC-complex antibodies over 10 years at a regional tertiary neurology centre determining which clinical/biochemical features were associated with antibody-positive disease.

We found a marked increase in the numbers tested, although the percentage positive remained low. Antibody titre was highest in central disease (p<0.001). Time from presentation to testing was shorter in those with VGKC-disease (p=0.01). Seizures were present in 11 (69%) of those with VGKC-disease versus 3 (18%) without (OR [95%CI] 10.3 [2.0-52.7], p=0.005). There was an inverse correlation between the antibody titre and serum sodium. A multivariate model selected seizures and hyponatraemia as predictive of VGKC disease (sensitivity 75% and specificity 82%); faciobrachial dystonic movements were specific but insensitive. Interestingly serum alkaline phosphatase was higher in those with VGKC-disease (p=0.016) and highest in those with peripheral disease (p=0.015). An ALP >70 u/L was strongly associated with antibody positivity (OR 4.11 [1.43-11.8], p = 0.007) with a sensitivity of 74.2%.

The presence of seizures, faciobrachial movements, and hyponatraemia should raise suspicion of VGKC-disease; alkaline phosphatase may represent a novel biomarker, particularly in those with peripheral disease.

**INTRODUCTION**

Voltage-gated potassium channel (VGKC)-complex antibodies were initially identified in association with peripheral nerve hyper-excitability.[1-5] They are now recognised to be associated with clinical syndromes of both the peripheral (PNS) and central (CNS) nervous system, causing neuromyotonia and limbic encephalitis respectively, or a combined phenotype termed Morvan’s syndrome.[6-9] This variation in clinical phenotypes is attributable to different antibody targets on the extracellular domains of neuronal cell membrane proteins, with antibodies directed against contactin-2 associated proteins (CASPR2) causing predominantly peripheral disease and those against leucine-rich glioma inactivated 1 (LGI1) associated with central manifestations.[10-14] Regardless of clinical presentation, early intervention is pivotal, particularly as timely immunotherapy can significantly improve outcome in those with CNS disease.[15-19]

However, an increasingly diverse clinical picture of VGKC disease is emerging. Several studies have implicated VGKC-complex antibodies in epilepsy, chronic pain, neuropsychiatric presentations and disorders of movement and autonomic function.[20-24] An expanding clinical spectrum, growing awareness and improved availability of serum assays has led to an increase in VGKC-complex antibody testing.[25] However, a positive serum VGKC-complex antibody assay does not preclude an alternative diagnosis.[22, 26-29]

This presents a challenge for neurologists considering testing for VGKC-complex antibodies, as excessive testing may be unnecessarily costly and potentially misleading, whilst not testing can lead to missed diagnoses. Therefore, we analysed the clinical and biochemical features of patients in whom VGKC-complex antibody testing had been performed to identify the clinical and laboratory features associated with antibody-positive disease.

**METHODS**

**Patients**

We conducted a retrospective case control study at the Walton Centre NHS Foundation Trust, a regional tertiary neurology centre in the UK, serving a population of 3.5 million people. The electronic laboratory database was screened over a ten year period (1stApril 2001 to 1st July 2011), to identify adults (>16 years) whose serum had been tested for VGKC-complex antibodies. Before 1st May 2005 sample processing was outsourced to a second laboratory; after this date all samples were processed internally.[29] A positive titre was defined as >100pmol/L on the Oxford assay and >85pmol/L on the commercial assay used at the Walton Centre. Because the number of negatives patients was many more than positives, and we wanted to analyse similar numbers, we randomly selected negative patients (1:1) with a random number generator.[30]

Both paper and computer-based clinical case notes were retrieved and examined by members of the team (AD, BO, EK, TS). Data were collected using a standardised proforma for demographics, clinical features, investigation results at the time of testing and the subsequent treatment, informed by syndromes of VGKC-complex antibody-associated disease.[6-8, 20, 22]

To reduce measurement bias, the data collection proforma was refined after an initial analysis of a subset of patients that was performed in duplicate by two members of the team (BO, BDM). Study methodology and analysis are presented in accordance with STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines.[31]

**Case definitions**

Antibody-positive cases were classified into clinical case definitions according to clinical features and VGKC-complex antibody titre as having CNS disease, PNS disease, both CNS/PNS disease, or as clinically-insignificant positive (Table 1). A case was defined as a clinically-insignificant positive if a positive VGKC-complex antibody titre was found, in the presence of a more likely alternative diagnosis, for example clinical features, electrophysiology and positive acetylcholine receptor antibody titres in keeping with myasthenia gravis. Where cases met one or more of these definitions, the most likely diagnosis as judged clinically was used, and any disagreements resolved through discussion with a more senior author (BDM, BO, TS). A case series of a subset of patients has been presented but not as a formal case-control study.[29]

**Statistical Methods**

Univariate statistical analysis was conducted using PRISM (GraphPad PRISM 2014). Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of clinical features were calculated and odds ratios with 95% confidence intervals generated. Chi-squared or Fisher’s exact tests were used to compare categorical data and the Mann-Whitney U test was used for continuous, non-parametric data. To minimise the potential for type I error, clinical features occurring in less than 10% of VGKC-complex antibody positive patients were excluded from the analysis. Hyponatraemia was defined by local laboratory protocol as sodium <133 mmol/L. The normal reference range for alkaline phosphatase (ALP) was 30-130 units/L).

Multivariate logistic regression analysis was performed using SPSS (SPSS v20 2012). The presence of VGKC-complex antibody-associated disease was used as a binary outcome measure. In order that missing data did not bias the multivariate analysis, variables that were not recorded in the majority of patients were excluded. Statistical significance was defined as *P*<0.05.

**RESULTS**

**Identification and clinical diagnosis**

Between 2001 and 2011, 1236 patients were tested for VGKC-complex antibodies, of whom 87 (7%) were positive (Figure 1). Over this period we identified a large increase in the numbers of patients tested. Between the first and second quarter studied the median (range) number tested per month rose from 3.5 (1-7) to 7 (3-13), p<0.0001; and between the second and third quarter studied this rose from 7 (3-13) to 15 (7-26), p<0.0001. Although there was an increase in the number tested per month between the third to fourth quarters studied this was not significant, 15 (7-26) to 16 (9-27), p=0.045. Despite this increased testing over these periods, the was no significant increase in the number of positive patients identified, and the percentage of positive patients remained low in each quarter studied (3.2%, 9.3%, 5.2%, and 7.0% respectively), and the percentage of those with VGKC-complex disease was low throughout the study period (Figure 2). Clinical information was available for 62 (71%) of patients with a positive antibody titre. Of these 35 (56%) met the clinical case definition for VGKC-complex disease; 13 (37%) had CNS disease, 17 (49%) had PNS disease and 5 (14%) had combined CNS/PNS disease. Twenty-seven (44%) met the clinical case definition for false positive and had a broad range of final diagnoses, many of which were other autoimmune conditions (Table 2a). In 24 (85%) false positive cases there were clear neuroimaging, neurophysiological, and/or cerebrospinal fluid abnormalities more consistent with an alternative diagnosis.

Patients meeting the clinical case definitions for VGKC-complex disease had significantly higher antibody titres than false positive cases (p<0.01) and those with CNS disease had the highest titre (p<0.001) (Figure 3). From the remaining 1149 VGKC-complex antibody negative patients, 60 were randomly selected as controls, for 50 of which clinical case notes were available (Table 2b). There was no statistically significant difference between those with VGKC-complex disease and antibody negative controls in median [range] age (50 [21-77] vs. 56 [18-82] respectively, p= 0.51) or the proportion who were male (21 [60%] vs. 29 [58%] respectively, p = 0.85).

**Demographics and presentation**

The median [range] time between presentation to tertiary services and antibody testing was significantly shorter in those with VGKC-complex disease compared with antibody negative controls (0 [-240 to 1013] vs. 29 [-4 to 2920] days respectively, p = 0.01). In addition, the duration of symptoms before antibody testing was shorter in those with VGKC-complex disease than antibody negative controls, although this was not significant (337 [30 to 21,360] vs. 547 [6 to 12,410] days respectively, p = 0.09). There was no difference in the proportion of patients tested as an inpatient between VGKC-complex disease and antibody negative controls, as the majority in both groups were tested as outpatients (9 (26%) vs. 12 (24%) respectively, p = 0.89).

**Clinical features**

There were several differences in the clinical features between the clinical case definition groups (Table 3). The proportion of patients with seizures was significantly higher in those with VGKC-complex disease (*p* = 0.005). The presence of uncontrollable faciobrachial movements was highly specific to VGKC-complex disease, although the proportion of patients with these symptoms did not reach statistical significance. Altered speech, fasciculations, and poor short-term memory were the most sensitive signs of VGKC-complex disease, although they lacked specificity.

There was an inverse correlation between antibody titre and serum sodium concentration in patients with VGKC-complex disease (r=-0.24, p = 0.02). However, there was no difference in the median [range] serum sodium concentration between patients with VGKC-complex disease and antibody negative controls (138 [124 to 145] vs 139 [126 to 147] mmol/L respectively, p= 0.45) and there was no difference in the proportion of patients with hyponatraemia between groups (4 (12%) vs 3 (8%) respectively, p = 0.66).

Interestingly, patients with VGKC-complex disease had a higher serum ALP than antibody negative controls (81 [52 to 136] vs 64.5 [30 to 133] u/L respectively, p = 0.016) (Figure 4). Patients with VGKC-disease were more likely to have an ALP greater than 70 u/L (OR 4.11 [95% CI 1.43-11.8), p = 0.007) and an ALP of greater than 70 u/L had a sensitivity of 74.2% for disease. However, only 14 had an ALP greater than 100 u/L and three were greater than 130 u/L. There was no difference between the proportions of patients in whom an underlying tumour was identified between the VGKC-complex disease and antibody negative controls, as this was only identified in a minority (3 [9%] vs 5 [10%] respectively, p = 0.82).

Multivariate logistic regression analysis showed seizures to be independently associated with VGKC-complex disease (OR 19.1 [95% CI 1.4-253.3], p = 0.025). A multivariate model incorporating hyponatraemia and presence of seizures demonstrated an improved association with VGKC-complex disease (sensitivity 75% and specificity 82%) compared with seizures alone (sensitivity 68.8% and specificity 82%).

**Subgroup analysis**

Analysis of the subgroups of VGKC-complex disease found that patients with CNS disease were more likely than antibody negative controls to have been tested as an inpatient (8 (62%) vs 12 (24%) respectively, p = 0.016) and were more likely to have abnormalities on cranial magnetic resonance imaging (MRI) (10 (83%) vs 8 (33%) respectively, p = 0.0095).

Patients with peripheral VGKC-complex disease had a lower median [range] age than antibody negative controls (47 [21-71] vs 56 [18-82] years, p = 0.04) and a shorter duration between presentation and testing (0 [240 to 435] vs 29 [0 to 2920] days, p= 0.02). This subgroup also had a significantly higher serum ALP than controls (81.5 [59-136] vs 64.5 [30-133]u/L, p = 0.015).

Of the patients with VGKC-complex CNS disease seven had an MRI scan, which was abnormal in cases. Three had bilateral hippocampal high signal, two had unilateral hippocampal high signal, one had non-specific high signal in the occipital lobes bilaterally, and one had minor changes of non-specific chronic white matter ischaemia.

**DISCUSSION**

VGKC-complex antibodies are associated with both central and peripheral nervous system disease, causing autoimmune encephalitis and neuromyotonia respectively, in part reflecting the antigenic target within the VGKC-complex.[16-18] However, overlapping presentations are recognised and the phenotype is expanding.[5-9, 23] This, in combination with increasing awareness and the wider availability of testing, has resulted in more patients being investigated for VGKC-complex antibodies. Early diagnosis is essential because early immunotherapy is associated with improved outcomes, particularly for CNS disease.[15,27] However, unselected testing of patients may result in a number of problems, including delays in establishing the correct diagnosis, over-diagnosis of VGKC-complex disease, and unnecessary expense. Previous studies have identified clinical features that seem to be common within VGKC-complex antibody positive cohorts, but no study has identified the usefulness of these to predict who will be positive, among all those tested.

Therefore, we undertook a retrospective case control study of patients who were tested for VGKC-complex antibodies by the attending neurologist at a tertiary centre to compare the clinical, laboratory, and neuroimaging features between those who were found to have VGKC-complex antibody disease and those who were negative and found to have an alternative diagnosis.

Over 10 a year period there was a marked increase in the number of patients tested. However, this was not associated with a significant rise in the number of positive patients identified; the percentage positive remained low throughout the study period. We also found that those tested early after presentation were more likely to be positive than those tested later on. This suggests that when the clinical suspicion of VGKC-complex disease is raised early it is more likely to be the diagnosis than in cases for whom the test is only ordered later down the line, once other tests have been found to be negative. We also found that the antibody titre was higher in those with VGKC-complex disease than false positive patients, and was highest in those with CNS disease, supporting previous findings.[11,12] Although the mechanism remains unclear, a higher proportion of those with CNS disease have antibodies directed against the LGI1 neuronal cell membrane protein component of the VGKC-complex.[11,12]The minority of samples in this study was tested for the individual antigenic component of the VGKC-complex targeted, therefore we analysed the overall positive cases, which is reflective of routine clinical practice. In our study the proportion of cases of clinically-insignificant positive titres was 44% which is consistent with findings from previous reports.[22,26]Also, around half of the false positive patients had a coexisting autoimmune disorder, most often myasthenia gravis and autoimmune polyneuropathies, as described.[22]

Those with antibody-associated disease were more likely to have seizures and the antibody titre had an inverse correlation with the serum sodium, as is often recognised in central disease.[8,11,15,19,22] However, as only a limited number of patients with VGKC disease have recurrent seizures in the long-term, our findings suggest that routine testing in such patients is less likely to be positive.32 A multivariate model identified seizures as the best parameter for distinguishing antibody positive disease from negative controls and the addition of sodium increased the sensitivity of the model. Hyponatraemia in VGKC disease may occur in up to 60% of patients, and has been attributed to a syndrome of inappropriate anti-diuretic hormone secretion.[6,7,11,12,18]Previous studies have demonstrated a normalisation of serum sodium with immunotherapy in conjunction with a corresponding decline in the VGKC-complex antibody titre, either because of direct action of the antibody or as an epiphenomenon.[18,32,33]Whilst our study did not find a higher proportion of patients with hyponatraemia in those with VGKC-complex antibody disease, the antibody titre did have an inverse correlation with serum sodium. Faciobrachial dystonic seizures, are described in those with central VGKC-complex antibody-associated disease, and are thought to be pathognomonic.[15,16,23] We confirmed that faciobrachial movements are highly specific for this condition, however the sensitivity was not great.

Intriguingly, the serum ALP was significantly higher in those with antibody-positive disease than controls, particularly in those with peripheral disease. ALP is in part produced by skeletal muscle and has long been recognised to be elevated in other diseases associated with fibrillation potentials and myopathy, such as dermatomyositis.[34] Although elevated levels of ALP have been reported in canine neuromyotonia, they have not been in human disease.[35] Therefore, as higher concentrations of ALP were found in those with VGKC-complex antibody-associated disease, particularly in those with peripheral involvement, serum ALP may be a biomarker of this activity.[36]

Taken together these finding suggest that when a neurologist is assessing a patient for whom VGKC-complex antibody-associated disease is in the differential, they are more likely to identify the condition in those who are tested earlier, particularly if seizures, faciobrachial movements, and hyponatraemia are present. Moreover, the serum ALP may represent a potential adjunctive biomarker, especially in peripheral disease. Nevertheless, this study reflects a cohort of patients in whom antibody testing was conducted in tertiary care and further case control studies in other centres are needed. Due to the retrospective nature of this study, where clinical features had not been described in the case notes the research team were unable to definitively record a symptom as absent or present.

**CONCLUSION**

This retrospective case-control study, which supports current understanding of VGKC-complex antibody-associated disease, has shown that earlier clinical suspicion; seizures and the degree of hyponatraemia were associated with higher rates of antibody positive disease. Faciobrachial movements are highly specific for VGKC-complex disease but not be sensitive. A raised serum ALP may be suggestive of VGKC-complex antibody-associated disease, particularly in the peripheral variant.

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**Table 1.** Clinical case definitions of patients tested for VGKC-complex antibodies in a tertiary care centre

|  |  |  |
| --- | --- | --- |
| **Serum Antibody Titre** | **Clinical Case Definition** | **Criteria** |
| Positive titre \* | VGKC-complex antibody-associated CNS disease (Limbic Encephalitis) | Seizures and/or neuropsychiatric symptoms. Absence of peripheral motor or sensory signs and symptoms |
| VGKC-complex antibody-associated PNS disease (Isaac’s syndrome/Neuromyotonia) | Clinical evidence of peripheral nerve hyperexcitability.Absence of central nervous signs and symptoms. |
| VGKC-complex antibody-associated CNS and PNS disease (Morvan’s syndrome) | Seizures and/or neuropsychiatric symptoms and clinical evidence of peripheral nerve hyperexcitability |
| Clinically-insignificant positive | Alternative diagnosis more likely |
| Negative titre \* | VGKC-complex antibody negative disease |  |

\*A positive titre was defined as >100pmol/L on the Oxford assay and >85pmol/L on the commercial assay at the Walton Centre.

**Table 2a.** Alternative diagnoses of VGKC-complex clinically-insignificant positive patients

|  |  |  |
| --- | --- | --- |
| **Diagnostic category** | **Diagnosis** | **Number (n)** |
| Immune/Inflammatory | Myasthenia Gravis | 6 |
|  | Acute Inflammatory Demyelinating Polyneuropathy | 5 |
|  | Chronic Inflammatory Demyelinating Polyneuropathy | 1 |
|  | Lambert-Eaton Myasthenic Syndrome | 1 |
|  | Stiff Person Syndrome | 1 |
|  | Multiple Sclerosis | 1 |
| Degenerative | Dementia | 1 |
|  | Multiple System Atrophy | 1 |
|  | Primary Lateral Sclerosis | 1 |
| Structural | Normal Pressure Hydrocephalus | 1 |
|  | Recurrent Right Petrous Apex Cholesteatoma | 1 |
| Vascular | Cerebrovascular Disease | 1 |
| Metabolic | Alcoholic Neuropathy | 1 |
| Other | Narcolepsy | 2 |
|  | Complex Regional Pain Syndrome | 1 |
|  | Focal Seizure Disorder | 1 |
|  | Restless Leg Syndrome | 1 |
|  | **Total** | 27 |

**Table 2b.** Final diagnoses of VGKC-complex antibody negative patients

|  |  |
| --- | --- |
| **Diagnosis** | **Number**  |
| No Diagnosis | 12 |
| Unexplained Peripheral Nerve Hyperexcitability Syndrome | 6 |
| Sleep Disorder | 4 |
| Benign Cramps | 4 |
| Dementia | 3 |
| Myasthenia Gravis/Lambert Eaton Syndrome | 3 |
| Multiple Sclerosis | 2 |
| Spinal Stenosis | 2 |
| CNS Malignancy in the absence of autoimmune antibodies | 2 |
| Epilepsy | 1 |
| Motor Neurone Disease | 1 |
| Blocked Ventriculo-Peritoneal Shunt | 1 |
| Polyneuropathy | 1 |
| Cerebral Amyloid Angiopathy | 1 |
| Superior Oblique Myokymia | 1 |
| Chronic Motor Tic Syndrome | 1 |
| Functional Disorder | 1 |
| Post-traumatic Brain Injury | 1 |
| Systemic Inflammatory Connective Tissue Disorder | 1 |
| Creutzfeldt-Jakob Disease | 1 |
| Post-Polio Syndrome | 1 |
| **Total** | 50 |

**Table 3.** Comparison between the symptoms and signs reported in patients with VGKC-complex antibody positive disease and VGKC-complex antibody negative controls

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Clinical features** | **Diagnostic group****CNS PNS CNS/PNS** | **Antibody positive cases (n=35)** | **Antibody negative controls (n=50)**  | **Sensitivity** | **Specificity** | **PPV** | **NPV** | **OR (95% CI)** | ***P*-value** |
| Seizures (n=33) | 10 | 0 | 1 | 11 | 3  | 68.75 | 82.35 | 0.79 | 0.74 | 10.27 (2.0-52.7) | 0.005 |
| Muscle Cramping (n=26) | 0 | 8 | 1 | 9  | 5  | 64.29 | 58.33 | 0.64 | 0.58 | 2.52 (0.52-12.30) | 0.25 |
| Fatigue (n=26) | 1 | 4 | 3 | 8  | 10  | 61.54 | 23.08 | 0.44 | 0.38 | 0.48 (0.087-2.65) | 0.40 |
| Paraesthesia (n=40) | 2 | 9 | 2 | 13  | 12  | 65.00 | 40.00 | 0.52 | 0.53 | 1.23 (0.34-4.46) | 0.74 |
| Headache (n=21) | 1 | 1 | 2 | 4  | 7  | 57.14 | 50.00 | 0.36 | 0.70 | 1.33 (0.21-8.29) | 0.76 |
| Hallucinations (n=18) | 4 | 0 | 1 | 5  | 2  | 23.81 | 77.78 | 0.71 | 0.30 | 1.09 (0.17-7.06) | 0.93 |
|  |  |  |  |   |  |  |  |  |  |  |  |
| **Signs/Investigation** |  |  |  |  |  |  |  |  |  |  |  |
| Uncontrollable faciobrachial movements (n=16) | 2 | 0 | 2 | 4 | 0  | 50.00 | 100.00 | 1.00 | 0.67 | 17.00 (0.74-391.7) | 0.077 |
| Confusion (n=36) | 8 | 0 | 2 | 10  | 11 | 71.43 | 50.00 | 0.48 | 0.73 | 2.50 (0.60-10.44) | 0.21 |
| Altered Speech (n=20) | 5 | 1 | 1 | 7  | 9  | 87.50 | 25.00 | 0.44 | 0.75 | 2.33 (0.20-27.6) | 0.50 |
| Fasciculations (n=40) | 0 | 10 | 3 | 13  | 16  | 76.47 | 30.43 | 0.45 | 0.64 | 1.42 (0.34-5.94) | 0.63 |
| Poor short-term memory (n=28) | 7 | 0 | 4 | 11  | 11  | 78.57 | 21.43 | 0.50 | 0.50 | 1.00 (0.16-6.08) | 1.00 |
| ALP > 70u/L (n=65) | 8 | 12 | 2 | 23 | 14 | 74.19 | 58.82 | 0.62 | 0.71 | 4.11 (1.43-11.8) | 0.007 |

(Sensitivity, specificity, PPV and NPV expressed as the proportion for whom data were available. ALP- Alkaline phosphatase; CNS- central nervous system; PNS- peripheral nervous system; PPV – positive predictive value; NPV – negative predictive value; OR – odds ratio; CI – confidence intervals).