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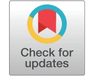
Complement system biomarkers in epilepsy



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ABSTRACT

Purpose: To explore whether complement dysregulation occurs in a routinely recruited clinical cohort of epilepsy patients, and whether complement biomarkers have potential to be used as markers of disease severity and seizure control.
Methods: Plasma samples from 157 epilepsy cases (106 with focal seizures, 46 generalised seizures, 5 unclassified) and 54 controls were analysed. Concentrations of 10 complement analytes (C1q, C3, C4, factor B [FB], terminal complement complex [TCC], iC3b, factor H [FH], Clusterin [Clu], Properdin, C1 Inhibitor [C1Inh] plus C-reactive protein [CRP]) were measured using enzyme linked immunosorbent assay (ELISA). Univariate and multivariate statistical analysis were used to test whether combinations of complement analytes were predictive of epilepsy diagnoses and seizure occurrence. Correlation between number and type of anti-epileptic drugs (AED) and complement analytes was also performed.
Results: We found:

1) significant differences between all epilepsy patients and controls for TCC ($p < 0.01$) and FH ($p < 0.01$) after performing univariate analysis.
2) multivariate analysis combining six analytes (C3, C4, Properdin, FH, C1Inh, Clu) to give a predictive value (area under the curve) of 0.80 for differentiating epilepsy from controls.
3) significant differences in complement levels between patients with controlled seizures (n = 65) in comparison with uncontrolled seizures (n = 87). Levels of iC3b, Properdin and Clu were decreased and levels of C4 were increased in patients with uncontrolled seizures.
4) no correlation was found between the level of complement biomarkers and the number of AEDs taken, but an association between some analyte levels and drug therapy was seen in patients taking sodium valproate, clobazam, and perampanel.

Conclusion: This study adds to evidence implicating complement in pathogenesis of epilepsy and may allow the development of better therapeutics and prognostic markers in the future. Replication in a larger sample set is needed to validate the findings of the study.
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1. Introduction

Epilepsy is a common disease; in England the prevalence of people with epilepsy who take anti-epileptic medication is 0.43–1.16% [1]. The 2017 International League Against Epilepsy consensus on epilepsy classification highlights the importance of defining aetiology, including immune causes [2]. Despite contemporary advances in neuroimaging and clinical genetics, the aetiology of epilepsy is still unknown in over a third of cases and a third of patients have seizures resistant to current antiepileptic drugs (AEDs) [3,4]. In these cases resective surgery is the best current option and can be

Abbreviations: FB, factor B; TCC, terminal complement complex; FH, factor H; Clu, clusterin; C1Inh, C1 inhibitor; CRP, C-reactive protein; ELISA, enzyme linked immunosorbent assay; AED, anti-epileptic drugs; CNS, central nervous system; WNRTB, Wales Neuroscience Research Tissue Bank; BSA, bovine serum albumin; PBS-T, phosphate-buffered saline containing 0.1% Tween; HRP, horseradish peroxidase; ROC, Receiver Operating Curve; AUC, area under the curve; MAC, membrane attack complex.

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curative, particularly in temporal lobe epilepsy; however; seizure recurrence occurs in up to half of patients within 5 years of operation [5]. Thus far precision medicine in epilepsy has been limited to the realm of the genetic encephalopathies [6]. A better understanding of aetiology would enable more effective treatment, targeted towards underlying pathogenic mechanisms [7].

Increasing evidence from experimental animal models and resected human brain tissue supports a role of the immune system in epilepsy [8]. At the population level there are prevalence correlations between auto-immune disorders and epilepsy; the risk of epilepsy is 3.8 times greater in people with any one of 12 autoimmune disorders and even higher in children with autoimmunity [9]. Systemic autoimmune disorders, such as systemic lupus erythematosus, have a neurological phenotype that includes a predeliction for seizures [2]. The most studied forms of immune epilepsy are Rasmussen's encephalitis, and the autoimmune encephalitidies associated with circulating antibodies [10]. Currently, primary immune-mediated epilepsies are recognised as neural autoantibody disorders affecting both cell-surface expressed proteins such as LGI1 and *N*-methyl-D-aspartate (NMDA) receptor, and intacellular proteins such as GAD [11]. A study of neural auto-antibodies in epilepsies of apparent unknown aetiology suggested that immune activation may explain up to 20% of non-paraneoplastic cases [12].

While the usefulness of autoantibody measurements, where present, as diagnostic biomarkers and treatment outcome predictors is robust, the mechanistic nature of the relation between autoantibodies and disease has yet to be elucidated in most cases. Positive responses have been reported for B cell ablation therapy using rituximab in some cases, suggesting a direct role of the autoantibodies in pathogenicity [13,14]. Furthermore, an absence of neural autoantibodies does not rule out the success of immunotherapies, or exclude a diagnosis of limbic encephalitis [13,15]. This lack of consensus and growing evidence of an immune/inflammatory component in epilepsy development makes it necessary to enlarge diagnostic and prognostic assessment to include other immunological biomarkers [16]. In response to this need, the involvement of different immune pathways in epilepsy pathogenesis is increasingly investigated in animal models and in humans [17–19].

One such pathway is the complement system, a major effector of innate immunity and an adjuvant of adaptive immunity. Complement comprises around 30 plasma and cell-surface proteins that interact with one another to induce a series of inflammatory responses involved in defence against infection [20]. Complement activation in the CNS is increasingly recognised to be associated with exacerbation and progression of tissue injury in degenerative and inflammatory diseases [21,22]. Dysregulation of the complement system in epilepsy has been observed both in human and animal studies [23–27]. For example, sequential infusion of individual proteins of the membrane attack pathway (C5b6, C7, C8, and C9) into the hippocampus of awake, freely moving rats induced both behavioural and electrographic seizures as well as neurotoxicity, suggesting a direct role for the complement system in epileptogenesis [28].

The aim of this study was to identify whether changes in the complement system occurred in the plasma of patients with epilepsy, and investigate whether plasma complement biomarkers could be used in diagnosis or stratification related to epilepsy syndrome and seizure control.

2. Materials and methods

2.1. Hospital records and samples

Patients were prospectively recruited through i) a secondary care adult epilepsy clinic, or ii) attendance for video telemetry as part of pre-surgical evaluation. The diagnosis of epilepsy was confirmed and classified according to current criteria [5], and presented in Table 1. No restriction in terms of epilepsy syndrome was made as part of inclusion criteria. All cases and controls included in the study gave informed consent. Ethical approval was granted through the Wales Neuroscience Research Tissue Bank (WNRTB). Detailed electro-clinical phenotyping, brain imaging, medication and seizure type and frequency at the time of sample collection, and for one year prior, were obtained from hospital records for all patients. Controlled epilepsy was defined as no seizure of any type in the past year. Relevant clinical variables and the results of investigations were entered into, and then extracted from, a customised clinical database (PatientCare) [29]. Plasma samples (157) were acquired from patients with epilepsy (106 focal epilepsy, 46 generalised epilepsy, 5 unclassified or single seizure) and tested alongside those from 54 healthy non-neurological disease controls sourced via the WNRTB. The controls included mostly staff or students consented for research and stored in the facility (WNRTB ethics REC# 14/WA/0073). The presence of autoantibodies was tested as part of routine clinical practice in cases where there was clinically assessed suspicion of autoimmunity; 19 were tested for anti-NMDA (one positive, uncertain significance), 21 were tested for anti-VGKC (none positive) and 4 were tested for anti-GAD (none positive). Five cases had co-existing thyroid disease; there were no other autoimmune conditions in the cohort.

Table 1
Distribution of diagnosis and aetiology of epilepsy for the study patients. For three patients more than one aetiology was indicated.

Diagnosis (syndrome)	Number of patients, n (%)
Temporal lobe epilepsy	61 (38%)
Juvenile myoclonic epilepsy.	29 (18%)
Frontal lobe epilepsy	21 (13%)
Focal epilepsy – not localised	23 (14%)
Idiopathic generalised epilepsy	8 (5%)
Epilepsy with generalised tonic clonic seizures alone	5 (3%)
Juvenile absence epilepsy	4 (2%)
Occipital lobe epilepsy	1 (0.64%)
Single epileptic seizure	1 (0.64%)
Unclassified epileptic seizures	4 (2%)
Aetiology	**Number of patients, n (%)**
Hippocampal sclerosis	30 (19%)
Focal cortical dysplasia	6 (3%)
Cerebral arteriovenous malformation	3 (1.9%)
Closed injury of head	3 (1.9%)
Dysembryoplastic neuroepithelial tumour	3 (1.9%)
Other structural abnormalities	18 (11%)
Idiopathic (presumed genetic) or Unknown	97 (61.78%)

2.2. Immunoassays

Eleven complement analytes were selected for this study, guided by reference to previous studies of complement biomarkers in epilepsy which have described increased serum levels or gene expression of C3, C4, C1q, iC3b and terminal complement complex (TCC), and availability of reagents and in-house assays [23,24,26,28,30]. The concentrations of nine analytes: iC3b, C1q, C3, C4, Properdin, Factor B (FB), Factor H (FH), C1 inhibitor (C1inh), and TCC were measured using established in-house enzyme-linked immunosorbent assays (ELISA) (Table 2). The marker set was chosen to interrogate classical (C1q, iC3b, C3, C4), alternative (Properdin, FB, FH, iC3b) and terminal (TCC) activation pathways. The remaining two analytes Clusterin (Clu), a complement cascade regulator and C-reactive protein (CRP), a benchmark of

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