Antibody biomarkers in CNS demyelinating diseases – a long and winding road

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Over several decades, studies sought potential markers to diagnose and to predict the clinical course of central nervous system (CNS) demyelinating disorders, especially in multiple sclerosis, acute disseminated encephalomyelitis and neuromyelitis optica spectrum disorders. Reliable biomarkers would ensure correct diagnoses, determine future disease evolvements, stratify patients for appropriate treatments and monitor disease activity and treatment effects – in summary, meet the longing for personalized medicine in these diseases. Out of a plethora of potential biomarker candidates antibodies have turned (again) into the scientific focus, due to pivotal immunological and neuropathological findings in the past 20 years. A major breakthrough and stimulus for further research was the identification of anti-aquaporin-4 antibodies in neuromyelitis optica. Various other myelin and non-myelin antigens were investigated in detail for diagnostic and prognostic purposes, such as antibodies to myelin oligodendrocyte glycoprotein or to the potassium channel KIR4.1. Further, the use of biopharmaceutical treatments in multiple sclerosis led to intense research activities to identify anti-treatment neutralizing antibodies and their clinical consequences. This review briefly summarizes the current knowledge on antibodies in the diagnosis, prognosis, disease and treatment monitoring of CNS demyelinating disorders.

Introduction – why is there a need for biological markers?

Inflammatory demyelinating central nervous system (CNS) diseases comprise multiple sclerosis (MS) and less prevalent disorders, such as neuromyelitis optica spectrum disorders (NMOSD) and acute disseminated encephalomyelitis (ADEM). These diseases usually show specific differences regarding age of onset, magnetic resonance imaging (MRI) and cerebrospinal fluid (CSF) features, clinical course, morbidity and, finally, treatment (Table 1). However, overlapping features in clinical, MRI and serum/CSF findings often make their diagnosis difficult, especially at onset referred to as the first demyelinating event. Thus, the diagnosis at this time point is of utmost importance to anticipate the potential further disease course and to decide a long-term disease-modifying treatment (DMT). At this stage the correct diagnosis is per se already of prognostic value as, especially in comparison to MS, ADEM has a monophasic course and NMO is a relapsing disease with the risk of high morbidity and mortality. In addition, the diagnosis also implies already a certain treatment stratification because, for example, NMO patients benefit specifically from B-cell/antibody targeting drugs and, in contrast, may worsen with DMTs approved for MS. However, the risk of future relapses in NMOSD is yet unpredictable due to the lack of a respective prognostic biomarker. This clinical dilemma accounts even more in MS: we are not yet able to predict the individual disease course, neither conversion from a clinically isolated syndrome (CIS) to relapsing–remitting MS (RRMS) nor from RRMS to chronic progressive MS (CPMS) nor disease activity or progression in general – again due to the lack of reliable biomarkers for monitoring...
the disease course and for prognosis. Disregarding the reflex that every CIS/RRMS patient is treated automatically with a so far not guaranteed fully effective DMT, such biomarkers would, without any doubt, enable personalized counselling, management and treatment with all its individual benefits from coping, planning and living with MS to treatment stratification and adjustment based on individual monitoring of the disease activity (and treatment effects).

How is it that antibodies engaged interest as biomarkers?

Humoral responses have been suggested since the early finding of elevated immunoglobulin (Ig) in the CSF of more than 90% of MS patients [1]. The intrathecal oligoclonal Ig response, which mainly involves IgG1 and IgG3 isotypes, is an important, albeit not pathognomic, diagnostic marker in MS [2,3]. In addition, humoral factors cause demyelination in rodent and primate experimental allergic encephalomyelitis, the animal model of MS [4,5]. Hence, various myelin and non-myelin antigens were suspected to be the target for a humoral immune reaction in CNS demyelinating diseases [6]. Amongst these, antibodies to the CNS-specific, highly immunogenic and myelin-surface-located myelin oligodendrocyte glycoprotein (MOG) were analysed most extensively [6,7]. Further, neuropathological studies identified that demyelination is antibody-mediated in a subgroup of MS patients [8,9]. The seminal finding of specific pathogenic antibodies against aquaporin-4 (AQP4) [10,11] separated NMOSD as a disease entity from MS, added substantially to current NMO diagnostic criteria [12], enabled specific treatment recommendations [13] and clinical trials, and boosted further antibody research in NMOSD, ADEM and MS. Finally, the use of biotherapeutics for treatment in MS opened a new field of antibody research, namely neutralizing antibodies (NABs), due to the immunogenicity of certain DMTs [14] such as interferon-β (IFNβ) products [15] and natalizumab [16].

Antibodies as diagnostic markers in CNS demyelinating diseases

The diagnostic significance of AQP4 antibodies in NMOSD is uncontradicted. However, two fields of scientific dispute emerged: first, as always with the initial use of various detection methods, different AQP4 antibody assays provoked discussions on their sensitivity and specificity; and, second, how to classify patients presenting with a clinical and radiological NMO phenotype but lacking AQP4 antibodies. The first problem was addressed by a multicentre approach for validation of these various AQP4 detection methods. The Eugene Devic European Network (EDEN), funded by an ERA-Net E-Rare grant, performed a study involving 15 European diagnostic centres which analysed 193 blinded NMO and control samples. The objectives were to assess the reproducibility of AQP4 antibody assays in Europe, to identify the best
method for AQP4 antibody detection and to ensure that this gold standard can be applied broadly. Results demonstrated that cell-based assays using the expression of recombinant AQP4 in human cells and visual scoring provided the most consistent results with high sensitivity and specificity [17]. However, a substantial number of phenotypic NMO patients are negative for AQP4 antibodies [18]. Screening different patient groups identified MOG antibody positive, AQP4 antibody negative patients with NMO and related disorders [19–30]. Although these NMO patients present at first glance similarly to those with AQP4 antibodies, they show distinct clinical features. Anti-MOG antibody positive NMOSD patients have an equal gender ratio, are often younger, mostly present with optic neuritis and experience a good recovery after relapse (Table 2).

A recent finding of serum and CSF IgG antibodies against a glial potassium channel KIR4.1 was suggested to aid the diagnosis of adult and paediatric MS [31,32]. However, subsequent studies [33–35] revealed controversial results, mainly unable to confirm the value of KIR4.1 antibodies for MS diagnosis [34,35]. Thus, more validation experiments are needed (and ongoing) to clarify the role of KIR4.1 antibodies in MS.

Despite promising animal data and a first indicator of a clinicopathological correlate for antibody-mediated demyelination [36] the relevance of antibodies directed against MOG in MS is still controversial due to variable frequencies of anti-MOG antibodies in MS patients and healthy controls [6,7] as well as the use of various antibody detection assays [6]. To identify the most reliable and valid anti-MOG test method two ‘ antimyelin antibody workshops’ were held in Innsbruck, Austria, in December 2004 and July 2005, and blinded validation experiments for various anti-MOG assays were done in 12 participating centres. The results clearly revealed that cell-based assays expressing MOG on their surface reliably detect antibody reactions against conformational and correct glycosylated MOG [37,38]. However, these antibodies against conformationally intact MOG are rarely found in adult MS patients, but occur in about one-third of patients with childhood acquired demyelinating syndromes such as CIS, MS and ADEM [24]. The first evidence for conformational dependent antibodies to MOG in ADEM came from a study using an assay based on self-assembling radiolabelled tetramers [39]. A humoral immune reactivity against conformational MOG has also been shown in paediatric MS with early disease onset and an initial ADEM-like presentation [40]. In 256 juvenile and adult subjects anti-MOG IgG titres using a cell based immunofluorescence assay were elevated in patients with ADEM in association with a younger age compared with patients with CIS, MS and controls [41]. Following studies confirmed and extended these previous findings [22,23,28–30]. Additional longitudinal analysis of serum anti-MOG IgG showed different temporal dynamics of serum antibody responses in ADEM, CIS and MS and indicated an association of a favourable clinical outcome in ADEM with a decrease

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Table 2 MOG antibodies in NMOSD (summary of published data [19–22,25–29,41])

<table>
<thead>
<tr>
<th>MOG-IgG positive</th>
<th>AQP4-IgG positive</th>
<th>Seronegative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>N = 118/790 (15%)</td>
<td>N = 370/790 (47%)</td>
</tr>
<tr>
<td>Age at onset (years)</td>
<td>28 (2–70)</td>
<td>41 (3–78)</td>
</tr>
<tr>
<td>Clinical subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMO</td>
<td>12.7% (15/118)</td>
<td>47.1% (180/370)</td>
</tr>
<tr>
<td>LETM</td>
<td>18.6% (22/118)</td>
<td>41.1% (152/370)</td>
</tr>
<tr>
<td>ON</td>
<td>49.2% (58/118)</td>
<td>9.5% (35/370)</td>
</tr>
<tr>
<td>Brainstem</td>
<td>1.7% (2/118)</td>
<td>0.8% (3/370)</td>
</tr>
<tr>
<td>ADEM (+ON/LETM)</td>
<td>17.8% (21/118)</td>
<td>0% (0/370)</td>
</tr>
<tr>
<td>All ON</td>
<td>71.2% (84/118)</td>
<td>58.1% (215/370)</td>
</tr>
<tr>
<td>Bilateral ON</td>
<td>35.9% (33/92)</td>
<td>9.7% (18/185)</td>
</tr>
<tr>
<td>LETM</td>
<td>44.9% (53/118)</td>
<td>89.2% (330/370)</td>
</tr>
<tr>
<td>Monophasic disease</td>
<td>48.2% (55/114)</td>
<td>28.7% (104/363)</td>
</tr>
<tr>
<td>Relapses</td>
<td>2 (1–16)</td>
<td>3 (1–20)</td>
</tr>
<tr>
<td>EDSS at nadir</td>
<td>4.5 (3–8.5)</td>
<td>6 (1–8.5)</td>
</tr>
<tr>
<td>EDSS at last FU</td>
<td>1.5 (0–8)</td>
<td>4 (0–10)</td>
</tr>
<tr>
<td>CSF OCB</td>
<td>4.7% (4/86)</td>
<td>15.7% (36/230)</td>
</tr>
<tr>
<td>CSF pleocytosis</td>
<td>35.9% (23/64)</td>
<td>28.5% (45/158)</td>
</tr>
<tr>
<td>Brain MRI lesions</td>
<td>50.0% (49/98)</td>
<td>41.3% (137/332)</td>
</tr>
</tbody>
</table>

ADEM, acute demyelinating encephalomyelitis; AQP4, aquaporin-4; CSF, cerebrospinal fluid; EDSS,Expanded Disability Status Scale; FU, follow-up; IgG, immunoglobulin G; LETM, long extensive transverse myelitis; MOG, myelin oligodendrocyte glycoprotein; MRI, magnetic resonance imaging; NMOSD, neuromyelitis optica spectrum disorder; OCB, oligoclonal bands; ON, optic neuritis.
in antibody titres over time [41–43], whereas antibody reactivity to MOG tended to be sustained in patients with suspected childhood MS [43]. In summary, there is increasing evidence for a discriminating potential of anti-MOG antibodies in ADEM versus MS and high-titre anti-MOG antibody responses in a subgroup of children with a CNS demyelinating disease (CIS/ADEM) [24].

Apart from the above mentioned detection of anti-MOG antibodies in patients with AQP4 negative NMOSD a recent study indicated that anti-MOG antibodies are also present in a few patients with demyelinating syndromes associated with anti-N-methyl-D-aspartate (NMDA) receptor antibodies [44]. However, even if data on the pathogenic roles of anti-MOG antibodies are limited [45], anti-MOG antibodies appear to define a new nosological entity within the spectrum of CNS demyelinating diseases.

**Antibodies as predictive markers in multiple sclerosis**

Research of the past decades sought to find predictors regarding conversion from CIS to RRMS, disease type and disease activity in terms of occurrence of future relapses and disease progression, especially the time point of conversion to CPMS.

Oligoclonal IgG bands in CSF are strong predictors for disease course progression in patients with MS at onset [46,47] – although, unfortunately, at many sites CSF analyses are not done routinely any more in suspected (mainly by MRI) CIS/MS cases. The presence of oligoclonal IgM bands in the CSF seems also to correlate with a worse MS disease course [48].

Increased humoral responses against myelin and non-myelin antigens are suggested to correlate with disease progression in MS [49]. Neurofilament protein subunits are potential CSF and serum biomarkers for disease progression in MS [50]. These may either contribute to or be due to axonal loss and the accumulation of disability in patients with disease progression. Further studies are now mandatory to standardize and validate different neurofilament assays and to determine the clinical meaningfulness in terms of prediction and monitoring disease progression in MS.

In patients with CIS there have been considerable efforts to identify an immunological prognostic marker for progression to clinically definite MS, because this conversion is unpredictable and needs long observation periods and repetitive MRI investigations. Initial results on the predictive value of anti-MOG antibodies in CIS patients were promising and sought to offer a potential tool in the management of CIS patients [51]. Subsequent studies were launched to reproduce these findings in CIS patients by using the same anti-MOG antibody detection assay but results were disappointingly inconsistent, ranged from highly significant to not significant at all [52–58]. These conflicting results may primarily reflect differences in study cohorts rather than methodological problems but, in conclusion, anti-MOG antibodies are not valuable to predict conversion from CIS to clinically definite MS in clinical routine practice. Further, it is very unlikely that the peptide-specific antibodies measured in these studies have a pathogenic role, but they may reflect tissue damage and ongoing neuroinflammation as has been demonstrated using peptide microarray analysis [59,60]. Recently it was demonstrated by using this method that paediatric MS patients developed a broader panel of antibody reactivities to CNS antigens at follow-up compared with disease onset, whereas corresponding CIS samples exhibited a contraction of the initial antibody response [61].

**Antibodies to monitor treatment efficacy and risks in multiple sclerosis**

Biopharmaceutical treatments are potentially immunogenic and thus induce binding and neutralizing antibodies with varying frequencies and clinical consequences in terms of therapeutic efficacy and potential adverse effects [14]. With the advent of recombinant human IFNβ products for treatment in RRMS a new era of biomarker research in MS begun. A number of studies demonstrated that the presence of NABs – with a frequency ranging from 7% to 42% according to the pivotal phase III clinical trials of three different IFNβ products – causes a reduction in IFNβ bioavailability as measured by reduced levels of bioactivity markers [62,63]. More importantly, as a consequence of neutralized bioactivity, persistently high-titre NABs, which usually occur 7–12 months after IFNβ treatment initiation, reduce the therapeutic efficacy of IFNβ in RRMS patients [62,63]. A European Federation of Neurological Societies guideline provides recommendations for the measurement and the therapeutic consequences of NABs against IFNβ [14].

In 2006 natalizumab was re-approved as the first monoclonal antibody therapy in neurology by the Food and Drug Administration and European Medicines Agency for active RRMS patients. Persistent NABs against natalizumab usually occur after the second or third infusion in only 6% of treated patients [15]. As a consequence, the biological activity and the clinical effect of natalizumab are completely blocked and, in addition, rare allergic reactions seem to be
linked to the presence of NABs to natalizumab. Thus, measuring for NABs is recommended after the first dose of natalizumab. However, the risk minimization plan for natalizumab requires more importantly a measure for anti-John-Cunningham virus (anti-JCV) antibodies, which indicate the risk for a rare but potentially fatal natalizumab associated adverse event, progressive multifocal leucoencephalopathy (PML) [64]. A serum anti-JCV antibody test was launched to improve PML risk assessment in clinical practice [65,66]. However, due to the fact that anti-JCV antibodies are prevalent in 50%–60% of humans [67], the request for a more specific risk stratification of anti-JCV antibody positive RRMS patients was met by analyses of the anti-JCV antibody index [68], which allows now a better, more individual benefit–risk evaluation in a given patient. However, further studies are necessary to define the longitudinal dynamics of anti-JCV antibody indices.

Conclusions

Out of a plethora of potential biomarker candidates antibodies have turned (again) into the scientific focus, due to pivotal immunological and neuropathological findings in the last nearly 20 years, but also due to pioneer collaborative approaches in the validation of various antibody detection methods and determination of the clinical meaningfulness of antibody biomarkers. A major breakthrough and stimulus for further research was the identification of AQP4 antibodies in NMOSD. Various other myelin and non-myelin antigens were investigated in detail for diagnostic and prognostic purposes, such as antibodies to neurofilaments and KIR4.1, which both require further studies. Anti-MOG antibodies seem now to be relevant in (paediatric) patients with ADEM, AQP4-seronegative NMOSD, but also in some cases of NMDA receptor encephalitis, thus tempting us to speculate that anti-MOG antibodies appear to define a new nosological entity within the spectrum of CNS demyelinating diseases. The use of biopharmaceutical treatments in MS led to intense collaborative research activities to establish valid assays for detection of NABs to IFNβ products and natalizumab and to determine their clinical consequences. Thus, monitoring for NABs as well as anti-JCV antibody (index) is now substantially important for treatment risk minimization strategies.

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Disclosure of conflicts of interest

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References


