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Aquaporin-4 IgG: Overview and future perspectives

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Abstract

The discovery of aquaporin-4 IgG in patients with demyelination is an exciting development. Initially associated with the Devic's phenotype, aquaporin-4 IgG has also been consistently found albeit less frequently in tumefactive disease, encephalopathies, classical MS and by one group in GBS. Curiously the cerebellum has the highest concentration of the target antigen, but remains the only part of the nervous system yet to demonstrate "characteristic lesions" with aquaporin-4 IgG. Moreover there is tantalising evidence that seropositivity is influenced by age, sex, and ancestral immunogenetic haplotypes. There is no exclusive clinical or radiological feature of seropositivity, and prospective cohorts universally find transitional cases. The use of teleological definitions is unhelpful and unscientific. Older detailed pathological studies have documented changes of both neuromyelitis optica and multiple sclerosis in the same individual, with both necrotising and classical lesions. Any hypothesis must accommodate the above observations, amongst other problematic findings. A logical initial conclusion is that demyelinating disease is a complex and extraordinarily heterogeneous process, and that aquaporin-4 IgG provides a new window into the disease. Crucially, the diagnostic and therapeutic implications of aquaporin-4 IgG can only be ascertained with evidence from rigorous prospective clinical study in different immunogenetic populations, and further pathological investigations are necessary.

The discovery of an antibody that was associated with NMO has reinvigorated research into multiple sclerosis (MS) and Devic's disease.[1] This antibody has subsequently been shown to bind the aquaporin-4 water transport channel. The detection of this antibody is giving us new insights into the spectrum of demyelinating diseases and has opened up fruitful avenues for further study. The discovery of this antibody has been of particular interest in the Asian region as optic and spinal involvement is more prominent in Asians than in Western case series. Furthermore a significant amount of attention has focused onto Asia and the clinical presentations of demyelination in the Asian region.

A number of basic questions arise in the interpretation of the antibody findings, and in our enthusiasm to embrace this new discovery we must analyse the results critically in order to move forward. Failure to address unresolved issues at an early stage will undoubtedly confound our understanding of significant findings, and ultimately retard our progress in this area. In this short presentation I will propose six brief questions and answers.

1. Does aquaporin-4 IgG help with phenotypic classification?

A great difficulty interpretating the presence of this antibody with a phenotype has been the elastic clinical definitions that are used with each manuscript. For example in 2007 neuromyelitis optica (NMO) was described as a "homogeneous disorder" and in the same paragraph described as a spectrum.[2] It is clear that a condition can either represent a spectrum, or it can be homogeneous, but it is not possible to be both. In this question I will focus entirely on phenotype, as it must be acknowledged that a number of different pathological processes can present with a similar phenotype, and it is not possible to address this second possibility at this point in time.

A study in France[3] found that aquaporin-4 IgG could be found in NMO, acute transverse myelitis, bilateral and/or recurrent optic neuritis and classical MS, which clearly indicates that the presence of this is not restricted to one clinical phenotype. Moreover in this article they attempted to further separate NMO from other conditions by applying the Paty MRI criteria in the NMO group, but the McDonald MRI Criteria[4] for MS,

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and despite this they still could not separate the two groups. They also stated "in definite NMO, from a clinical point of view, the test is of limited interest".[4] The antibody however did associate with the length of the lesion.

Attempts to criticise statements regarding phenotypic restriction of the antibody have been vigorously attacked in the past. Kikuchi and Fukazawa[5] pointed out some of the obvious circular reasoning in this regard, but were vigorously attacked in a reply to this letter. Weinshenker and colleagues stated, "they reject our proposal that NMO and optic-spinal MS are identical on the basis of serological findings". "… NMO-IgG detection allows early distinction".[6] They also stated that there were "no important differences between NMO and Japanese optic-spinal MS".[6] I note however that this point of view is now rejected by the same authors (BG Weinshenker, personal communication, 2008 PACTRIMS Meeting). The same response[6] also addressed the presence of brain lesions in the majority, as opposed to no brain lesions in all.

Very interesting data were found when aquaporin-4 IgG was tested in children with demyelinating disease.[7] In this article cerebral presentations of demyelinating disease, such as encephalopathy and acute disseminated encephalomyelitis occurred in 16% of children. This compares with only 9% of children with NMO by 1999 clinical criteria.[8] Indeed the percentage of patients presenting with cerebral presentations such as encephalopathy, diplopia and vomiting was roughly comparable to the percentage of patients presenting with transverse myelitis alone. This data does not suggest a highly restricted phenotype in aquaporin-4 IgG positivity.

Finally the comprehensive manuscript from Professor Jun-ichi Kira's group[9] also showed aquaporin-4 IgG being detected in patients with classical MS, both with and without longitudinally extensive spinal cord lesions, and with and without cerebral MRI changes meeting the Barkhof criteria. The most interesting data however was found when comparing those patients clinically defined as optic-spinal MS. These patients had a higher rate of sero-positivity of aquaporin-4 IgG than other groups, but interestingly the sero-positivity in optic-spinal MS cases with longitudinally extensive spinal cord lesions was twice as high in patients who satisfied cerebral Barkhof MRI criteria, than those patients in whom Barkhof criteria for cerebral MRI were not met.

A recent Japanese study[10] also found that aquaporin-4 IgG was found not only in classical MS as well as NMO cases, but it was also found in 3 of 52 patients with Guillain-Barre syndrome.

The conclusion for the first question therefore, is that aquaporin-4 IgG is not invariably associated with a restricted clinical phenotype.

2. What is the specificity and sensitivity?

Most manuscripts quote very high specificity and sensitivity for the detection of this antibody, but it is clear that the definition has been somewhat elastic. A recent article quoted a 99% sensitivity and a 90% specificity in distinguishing NMO from MS presenting with optic nerve and spinal cord involvement.[11] A great difficulty here is that different manuscripts used different definitions. In 2004 the highest specificity was established by excluding all patients with cerebral lesions at presentation.[1] These high specificities and sensitivities were also applied to Japanese patients with optic-spinal MS, using the definition of no evidence of clinical disease outside the optic nerve or spinal cord (excluding minor brainstem symptoms). Very soon however it became apparent, as had been observed in Asian cases over many years, that cerebral lesions were not rare, but common.[12] Including patients with brain diseases however did not appear to alter the specificity or sensitivity. It appears contradictory that if all patients with brain lesions are excluded in some classifications of specificity, whereas brain lesions occur in the majority with another classification, that the use of the term "specific" for a phenotypic definition becomes difficult to justify. NMO is indeed an empirically defined syndrome for which there is no definitive diagnostic test.[13] Proponents for aquaporin-4 IgG however would argue that aquaporin-4 IgG positivity is the definition, irrespective of phenotype. Using this circular logic, positivity of the antibody would invariably be specific and vice versa. As we have seen in other manuscripts[3,10] it is self-evident that aquaporin-4 IgG is found in a wide variety of clinical situations. This also includes patients with tumefactive brain lesions[12], recurrent optic neuritis and recurrent transverse myelitis, and in acute disseminated encephalomyelitis in the complete absence of any involvement of optic nerve and spinal cord.[7] Even when one attempted to separate the various clinical groups, such as by using Paty criteria for negative brain MRI and Barkhof criteria for a positive brain MRI, they still overlap considerably.[3,10]

In truth, most authors found much lower sensitivity than the quoted 90-99%. A recent abstract presented at the World Congress for Treatment and Research in Multiple Sclerosis found that only 8 of 74 patients defined as NMO or high risk longitudinally extensive transverse myelitis or recurrent optic neuritis were positive for the antibody, and all were women.[14] At the PACTRIMS Meeting we have also heard that only 1 of 11 patients with NMO in Singapore[15] and only 4% of patients with an NMO spectrum in India as tested by the Mayo Clinic[16], and only 1 in 20 patients with longitudinally extensive spinal cord lesions in Australia are positive for this antibody.[17]

The conclusion for question two is that current methods of defining specificity and sensitivity are meaningless as they are currently reported, as it is seen in an extraordinarily heterogeneous variety of clinical presentations and situations, with markedly different rates of positivity, even when the studies are done by the same laboratory but in different populations. The loose use of the term "NMO spectrum" to refer to any form of demyelinating disease in adults and children in whom the Aquaporin-4 IgG antibody is detected must be avoided if progress is to be made in this area.

3. Does aquaporin-4 IgG positivity help with prognosis?

There is conflicting evidence as to the role of aquaporin-4 IgG in determining prognosis in demyelinating disease. At the World Congress for Treatment and Research in Multiple Sclerosis 2008 in Montreal, aquaporin-4 IgG positivity was found in 11 of 460 patients.[18] There was no difference in either baseline characteristics or clinical outcomes in aquaporin-4 IgG positive patients versus aquaporin-4 IgG negative patients. Other publications however have shown that aquaporin-4 IgG positivity may associate with earlier relapse. In optic neuritis positivity was stated as predicting the outcome of recurrent optic neuritis.[19] In long term follow up one of 15 seronegative patients developed MS versus 6 of 12 developing further demyelinating events over a median of 8.9 years of follow up. There were some concerns however with the follow up study given that "data for patients evaluated elsewhere were ascertained only for sero-positive RAN patients … in the course of contacting physicians …".[19] Similarly 4 sero-positive and 7 seronegative patients had optic neuritis relapses after NMO-IgG testing (P=0.69).[19] Therefore these results were not as conclusive as one would hope. The data however is stronger in the context of recurrent longitudinally extensive transverse myelitis.[20] In a study of 23 patients with longitudinal extensive transverse myelitis one of 14 who were NMO-IgG seronegative developed recurrent myelitis whereas 5 of 9 NMO-IgG sero-positive patients developed myelitis. Therefore antibody testing may be of value in this clinical context, although these numbers fall well short of the 90% or more sensitivity and specificity, which were frequently quoted for "NMO spectrum". These issues were discussed in an editorial.[21]

A European study attempted to correlate titres of aquaporin-4 to risk of relapse.[22] In this manuscript they found that in some cases titres of aquaporin-4 IgG rose prior to relapse. This detailed report however made less of the fact that 3 of 8 cases in their group had very high titres of aquaporin-4 IgG but did not suffer any relapses, and a further 2 of their 8 patients developed relapses whilst their titres of aquaporin-4 IgG were falling, giving 5 of 8 instances where there was a negative association of aquaporin-4 IgG and relapses.[22] Similarly many neurologists have cases with very long and benign histories that have high titres of aquaporin-4 IgG antibody (JI Kira, SL Galetta, personal communication). Finally in the French experience[3], when comparing aquaporin-4 IgG positive and negative patients, they found no significant differences at all with respect to age and onset of disease, annualised relapse rate, brain MRI findings and CSF abnormalities. Similarly there was no difference between the two groups considering the symptomatology of their inaugural episode.

The conclusion for Question 3 therefore, based on the evidence to which I have referred to above, is that aquaporin-4 IgG is an unreliable predictor of prognosis. Whilst in some instances the presence of the antibody has been associated with increased relapse rate, in others there has been no association and indeed extremely benign cases despite high titres of aquaporin-4 do exist.

4. Does aquaporin-4 IgG explain lesion distribution?

We have already addressed in Question 1 that there is a poor correlation of the presence of aquaporin-4 IgG antibody with phenotype. It is often stated however that the distribution of lesions in aquaporin-4 IgG positivity is explained by the distribution of the aquaporin-4 water channel.

Aquaporin-4 water channel however is widely distributed throughout the body, and particularly in the stomach and the kidney where no blood tissue barrier exists[23], and where despite antibody binding there is no evidence of pathological damage at those sites. Within the central nervous system aquaporin-4 is ubiquitous but particularly so in the periventricular areas and the cerebellum, which also happen to be the areas where classical MS lesions occur.[23] It is interesting to track the evolution of diagrams of the distribution of aquaporin-4. In 2003 diagrams clearly show high concentrations of the antigen within the cerebellar folia and cerebellar hemispheres as well as along the lateral ventricles and the spinal canal.[23] However, a diagram in a manuscript from 2006 has most of the aquaporin-4 removed from the cerebellum[24], and a manuscript in 2007 by the same authors shows aquaporin-4 IgG reactivity removed from the cerebellum entirely.[2] There is strong evidence therefore for selective interpretation and editing of the data. It has also been stated that there are "characteristic brain lesions", particularly in areas involving grey matter and diencephalic areas, but it should be noted these were only seen in 8 of 120 cases[12], and similar lesions can be seen in patients who are negative for both aquaporin-4 IgG and NMO-IgG (JI Kira, I Sutton, AG Kermode, personal communication). Similar comments are made regarding the central preponderance for MRI lesions in aquaporin-4 IgG positive patients, but it is recognised by all neurologists that both central and peripheral spinal lesions may be seen in the same patient, whether they are aquaporin-4 IgG positive or negative. The paradox of the distribution and the typical optic spinal predominant location of NMO lesions despite the widespread distribution has been noted by many authors. Roemer *et al* stated "the widespread expression of aquaporin-4 in the brain is paradoxical in face of the typically optic spinal predominant locations of NMO lesions and predilection for brain stem".[25]

The conclusion for Question 4 must therefore be that aquaporin-4 IgG does not explain the lesion distribution in NMO.

5. What is the evidence for pathogenicity of aquaporin-4 IgG?

This question can be addressed from a logical perspective. The first is that the presence of an antibody does not necessarily prove its pathogenicity. In classical Latin this error of interpretation is stated thus "*cum hoc, ergo, propter hoc*". This can be translated as "with this, therefore, because of this". The second is the logical error of "*post hoc, ergo, propter hoc*" which may be interpreted as "it follows this, therefore, it is because of this". Neither the presence nor the sequence of events with detection of an antibody necessarily proves that it is pathogenic. Nevertheless the apparent association of this antibody with demyelinating disease certainly raises an important question regarding pathogenicity. Numerous preliminary pathological studies have shown a number of interesting changes in NMO lesions, which include loss of aquaporin-4 immunoreactivity and "rosettes" containing mixed IgM within lesions. It should be emphasised however that loss of aquaporin-4 can also be seen in MS lesions. "We also observe complete loss of Aquaporin-4 immunoreactivity in inactive MS lesions sampled from both the acute and chronic phases of the disease".[25] Similarly the immunoglobulin deposition seen in NMO is predominantly mixed IgM, and is not IgG specific for Aquaporin-4. Identical NMO syndromes and central nervous system lesions are also seen in the complete absence of detectible aquaporin-4 IgG reactivity. Further observations which should temper our enthusiasm for accepting aquaporin-4 IgG as pathogenic is that robustly high titres of aquaporin-4 IgG in animals do not cause any detectable disease by themselves. To produce disease in the rodent it is necessary to induce disruption in the blood brain barrier via similar mechanisms as in EAE, therefore at best aquaporin-4 IgG in these circumstances could be a modulator of disease. To date passive transfer experiments have also been unhelpful in furthering our understanding as to whether or not aquaporin-4 IgG is pathogenic. Nevertheless, we should consider a variety of possibilities for the clinical and pathological relevance of aquaporin-4 IgG, as it may well be a surrogate disease marker of some other process, or an important modulator of disease, but the evidence for it as a primary pathogenic antibody is lacking at this point in time.

The conclusion for Question 5 must be therefore that direct evidence for aquaporin-4 IgG pathogenicity is absent at this point in time and that further study is needed.

6. Where do we go from here?

It will be necessary to investigate the pathogenicity of aquaporin-4 IgG both in vivo and in vitro, and be careful that we do not over interpret our findings.

It will be necessary to objectively ascertain and study in a prospective and longitudinal fashion patients with demyelinating syndromes both in Asia and in the West, and combine this with appropriate MRI and serological studies. The genetic and epidemiological aspects of each population must also be considered, as there is already compelling evidence for different rates of positivity in different population groups with the same clinical syndromes. Finally, as in every other immunological assay, it will be necessary for the assays to be standardised both with respect to their titre as well as to their biological specificities. The variety of methodology available for testing aquaporin-4 has already demonstrated significant discrepancies between different assays.[10] It will also be important for us to recognise the significant gaps in our understanding of aquaporin-4 IgG sero-positivity in demyelinating syndromes, as over only four short years we have seen two strongly held beliefs, that of an absence of brain lesions and the belief that optic-spinal MS is identical to NMO (vigorously defended as dogma), only for the opinions to be completely reversed thereafter. As clinicians and scientists we are familiar with making clinical decisions on the basis of imperfect data, but we must be prepared to acknowledge the limitations in our evolving knowledge.

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