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# Treatment Modalities in Multiple Sclerosis and Nmosd: the Effect of Anti AQP4 Antibody.

KEYWORDS: AQP4, NMOSD, MS, treatment response

*** Dr. Suhailah Abdullah**

Division of Neurology, Department of Medicine, University Malaya Medical Centre, 59100 Kuala Lumpur, Malaysia * Corresponding author

**ABSTRACT** ***Objective****: to determine the effect of AQP4 status in relation to treatment response, in patients with IIDDs presented to our centre.*

***Method****: Retrospective analysis of treatment response in patients with IIDD in relation to the AQP4 status.*

***Results****: 59/ 102 (57.84%) were on at least one treatment. β-Interferon was associated with the highest rate of treatment failure in the seropositive group, 24/ 24 (100.00%) as compared to 2/ 11 (18.18%) in the seronegative group (p< 0.005), followed by mitoxantrone. Oral Azathiophrine was the second common treatment modality. This was associated with significant reduction in the number of relapse (p= 0.008). However, 11/ 23 (47.83%) developed intolerance to the treatment in the form of persistent neutropenia, pancytopenia, acute hepatitis, and 1 case with locally invasive breast carcinoma. 6/ 8 (75.00%) experienced no relapse after initiation of IV Rituximab, associated with improvement in term of relapse rate and baseline EDSS (p= 0.003 and 0.022 respectively).*

***Conclusion****: The status of anti AQP4 antibody is important in determining the choice of disease modifying therapy.*

**Introduction:**

Idiopathic Inflammatory Demyelinating Diseases (IIDDs) of the Central nervous system (CNS) have a broad spectrum of neurological presentations, in which Multiple Sclerosis (MS) is the most common form. Neuromyelitis optica (NMO) was previously thought to be a monophasic disease and a spectrum of MS, with a predilection for optic nerves and spinal cord. However, the discovery of anti Aquaporin 4 (AQP4) antibody or NMO Immunoglobulin (IgG) distinguished NMO as a separate disease from MS (Sato et al., 2013; C Trebst et al, 2014). Recently, the term Neuromyelitis Optica Spectrum Disorders (NMOSD) was introduced, which includes clinical syndromes and MRI findings related to area post rema, other brainstem, diencephalic or cerebral presentations, in addition to seronegative opticospinal cases with optic chiasmal involvement and longitudinally extensive spinal cord lesions (Wingerchuk DM et al., 2015).

Even though there has been a rapid advancement in term of treatment modalities for MS, the "standard care" for NMOSD, especially seronegative cases are less well defined. This was in part due to the lower prevalence of NMOSD as compared to MS in the West. More importantly, it was discovered that some disease modifying treatment for MS may exacerbate NMOSD, resulting in disabling relapses and disability progression. The aim of this study is to determine the effect of AQP4 status in relation to treatment response, in patients with IIDDs presented to the University Malaya Medical Centre, Kuala Lumpur, Malaysia.

**Methodology:**

We retrospectively reviewed a total of 102 patients presented with IIDDs to the Neurology unit in the University Malaya Medical Centre, Kuala Lumpur, Malaysia, from 2005 to 2015. Based on the clinical presentations, patients were classified into 3 major groups that include Classical Multiple Sclerosis (CMS) according to Mc Donald's criteria 2005 and 2010, opticospinal (OS) and brainstem involvement (BS). The treatment data was obtained from the medical records by a neurologist (SA). Treatment failure was defined as more than 1 major relapses after 6 to 12 months of treatment initiation, or disability progression as measured by increased of ≥ 1 EDSS in one year, or treatment termination due to adverse event. Patient was tested for serum anti-AQP4 antibody using the Indirect Immunofluorescence Test (IIFT) cell based assay (EUROIMMUN IIFT, Germany). Statistical analysis was done using the SPSS version 20. Values of $p < 0.05$ were considered as significant.

**Results:**

We retrospectively reviewed 102 patients presented to our centre from 2005 until 2015 with the diagnosis of IIDDs. Relapsing remitting disease was the commonest disease course (77/ 102, 75.49%), with 25/ 102 (24.51%) presented as first neurological deficit. Two patients refused blood taking for anti AQP4 antibody. In the remaining 100 patients, serum anti AQP4 antibody was detected in 53% of patients, with up to 86.80% of all the presentation perceived to be characteristic of NMOSD (opticospinal 49.06%; $p < 0.004$, transverse myelitis 30.19% and optic neuritis 7.55%). On the other hand, CMS was the most common presentation in the AQP4 negative group (57.45%; $p < 0.001$), followed by transverse myelitis (19.14%), opticospinal involvement (10.64%) and optic neuritis (8.51%)(Table 1).

In total, 59/ 102 (57.84%) were on at least one treatment modality throughout their neurology follow up. Thirty two patients were on at least 2 treatment regimes due to treatment failure (Table 2). Eight patients (13.56%) were subsequently loss to follow up. Among the remaining 43 patients not on treatment, 16 presented with first time neurological event, and 19 defaulted subsequent follow up.

Table 3 illustrated the breakdown of treatment modalities between the AQP4 positive and negative groups. Subcutaneous B-Interferon (βIFN) was the most commonly prescribed medication (42 patients), followed by oral Azathiophrine (27 patients), intravenous Rituximab (8 patients) and Mycophenalate myfotil (MMF, 4 patients). Other utilized treatment includes intravenous Alemtuzumab, cyclical intravenous Immunoglobulin (IVIG), oral fingolimod and leflunamide (1 patient each).

Volume : 6 | Issue : 8 | August 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

When the treatment modalities were divided according to their AQP4 status, β-Interferon was associated with the highest rate of treatment failure in the seropositive group, 24/ 24 (100.00%) as compared to 2/ 11 (11.76%) in the seronegative group (p< 0.005), followed by mitoxantrone.

**Treatment failure**

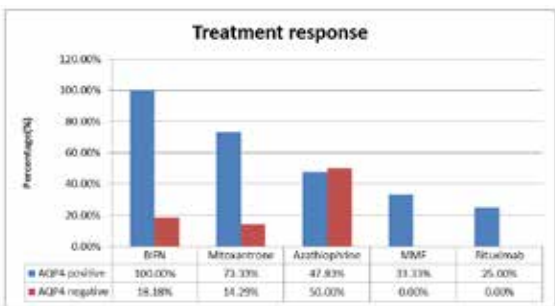


Figure 1 Oral Azathiophrine was the second common treatment modality. However, 11/ 23 (47.83%) of patients in the seropositive group developed intolerance to the treatment in the form of persistent neutropenia (lowest white blood cell count of 2.2 x $10^9$), pancytopenia, acute hepatitis, and 1 patient was diagnosed with locally invasive breast carcinoma (Table 4). Only 1/ 3, (33.33%) patient on oral mycophenalate myfotil developed neutropenia associated with dizziness. Two out of 8 patients on intravenous Rituximab developed at least three disabling relapses within a year after treatment, with poor response to both intravenous methylprednisolone and plasma exchange. Intravenous Alemtuzumab was used in one seropositive patient, but it was also associated with disabling relapses. One seronegative patient on cyclical IVIG was relapse free during the 1 year treatment period, but a repeat MRI of the brain done 6 months after completion of treatment showed 6 new T2 hyperintense lesions. The patient on oral leflunamide developed a cerebellar relapse 17 months after treatment initiation, and one patient on oral fingolimod self-stopped the treatment after 4 months due to financial constraint.

**Discussion:**

**B-Interferon (βIFN)**

βIFN is a standard treatment for Relapsing remitting, and to some extent secondary progressive MS. It belongs to the type I interferons (IFN-I) group that naturally produced cytokine, inhibits leukocytes proliferation, antigen presentation and T-cell migration across the blood brain barrier (M. Krumbholz et al., 2008; N Collongues et al., 2011). Several class III studies had shown that treatment with βIFN in NMOSD patients resulted in an increased in the relapse rate and disability (Uzawa et al. 2010; Tanaka et al. 2009; Saida et al. 2005). This was also supported by our study, in which 24/ 24 (100.00%) seropositive patients developed treatment failure with βIFN (p< 0.005). It was postulated that treatment with βIFN resulted in a strong up-regulation of B cell activating factor (BAFF) in the neutrophils, fibroblasts and astrocytes. Hence, stimulating pathogenic B cells activity, auto antibody production and worsening of CNS inflammation (M. Krumbholz et al., 2008). In the seronegative group, 1 out of 2 patients with treatment failure presented with recurrent ON and long extensive transverse myelitis, typical of NMOSD.

**Mitoxantrone**

Mitoxantrone was developed in the late 1970s, originally designed as an anti-neoplastic agent. It also has a potent immunosuppressive property targeting proliferating immune cells which includes B and cytotoxic T cells, and reduces the secretion of tumor necrosis factor-a (TNF a) and inter-leukin 2 (IL-2) (O Neuhaus et al., 2006, 2011). Mitoxantrone infusion was the second option of treatment in both seropositive and seronegative patients in our cohort of patients. This was due to financial constraint and aggressive nature of relapses in some patients. In view of the smaller built of Asian patients in general (in our cohort: average height 1.5 to 1.6 meter, weight 50 to 60 kg), induction therapy was only given in aggressive disease, and patients were given a standard three monthly mitoxantrone infusion of 5 to 10 mg, to a cumulative dose of 80 to 100 mg. Thirteen patients (10 seropositive, 3 seronegative) completed the treatment, with a mean follow up of 6.38 years (range 4 to 10 years). It was noted that the number of relapses decreased after a cumulative dose of 25 to 30 mg were reached, and the majority of patients remained relapse free thereafter, until a targeted dosage was reached. This has lead to the addition of oral prednisolone 10 mg daily during this sub-therapeutic interval which resulted in absence of acute relapses in the seropositive group. However, relapses recurred within the first 2 years of treatment completion. This might be explained by the fact that mitoxantrone can only persist in the body up to 272 days after cessation of treatment (Stewart DJ et al., 1986). Thus, the urge to find a more sustainable and affordable treatment option. Even though there was only 1 documented treatment failure in the seronegative group, only 3 patients had completed treatment within the last 6 months. Hence, the long term outcome remained to be seen. The major adverse event associated with treatment with mitoxantrone, namely persistent neutropenia, myelosuppression and cardiomyopathy, were not observed in our patients (JJ. Marriott et al., 2010; E Le Page et al, 2011). This was likely to be explained by the lower cumulative dose of treatment per body surface area.

**Azathiophrine**

Azathiophrine is a steroid sparing immunosuppressive drug that has been found to be effective in preventing relapses in NMOSD at the dose of 2 to 3 mg/ kg body weight. Twelve patients (3 were treatment naïve) tolerated the treatment with a mean follow up 1.33 years (range 1 to 3 years). Even though there was no statistical difference in term of post treatment EDSS, it was associated with significant reduction in the number of relapse (p= 0.008) (Table 5). Adverse events were observed in 11 patients, resulting in termination of treatment. However, testing for Thiopurine methyltransferase enzyme activity (TMTP) was not available to stratify patients into low or high risk groups (C Trebst et al, 2014). One AQP4 negative patient presented with optic neuritis and recurrent transverse myelitis with LESCLs on MRI, was highly steroid responsive and responded well to oral azathiophrine. Hence, a likely diagnosis of seronegative NMOSD instead of opticospinal MS.

**Rituximab**

Rituximab is an anti CD 20 monoclonal antibody against the B-lymphocytes that resulted in B-cell apoptosis (Collongues et al., 2011; Ip VHL et al., 2012). Previously limited to the treatment of hematological malignancy, it is currently shown to have a broader spectrum of efficacy in the treatment of auto immune diseases like Systemic Lupus Erythematous, polymyositis and myasthenia gravis. The efficacy of Rituximab has also been extended to the treatment of RRMS and NMOSD (A Jacob et al., 2008; N Collongues et al., 2011; He D, 2011).. The dosage of Rituximab used in our centre was 1g per 4 hour infusion

Volume : 6 | Issue : 8 | August 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

on day 1 and day 15, at a 12 months interval. Nevertheless, this modality of treatment was expensive with a total cost of RM $22,000 per 2g infusion. Monitoring for CD $19^+$ or CD $27^+$ B- cells was not available at our centre. Hence, the decision on the annual infusion was made based on the knowledge that restoration of the B-cell repertoire generally takes 9 to 12 months from the last Rituximab infusion (Dass et al. 2008). In our cohort of patients, 6/ 8 (75.00%) experienced no relapse after initiation of IV Rituximab given at a yearly interval. This was also associated with improvement in term of relapse rate and baseline EDSS (p= 0.003 and 0.022 respectively) (Table 5). To date, our longest follow up for patient treated with IV Rituximab was 4 years (range 1 to 4 years). The previously reported infusion related adverse event and increased risk of infection were not seen in our cohort of patients. There was no case of Progressive Multifocal Leukoencephalopathy (PML) or malignancy observed.

In summary, the status of anti AQP4 antibody is important in determining the choice of disease modifying therapy, especially in the South East Asia region where financial constraint dictate treatment option and adherence. βIFN was associated with 100.00% treatment failure in the seropositive group and should be avoided. Mitoxantrone was a cheaper option and can be used in both groups of patients, provided that the side effect profile is closely monitored. However, the preventive effect was not sustainable beyond 2 years. Azathiophrine is cheap, easily available and significantly reduces the number of relapse. Provided that the side effect profiles are being closely monitored, it should be used as a first line treatment in the seropositive group. This will prevent delay in treatment initiation due to financial constraint; therefore reduce the occurrence of disabling relapses and disability progression. Treatment with Rituximab was associated with significant improvement in term of number of relapses and EDSS. A 12 monthly intervals in between infusions appeared to be well tolerated in term of efficacy and safety. Nevertheless, the treatment was expensive, and should be used as a second line option. On the other hand, the approach to seronegative NMOSD is still a matter of debate, and should be tailored as per patient basis. We acknowledge the limitations of this study, being retrospective and descriptive in nature. However, this is the first study done in Malaysia comparing the effect of anti-AQP4 antibody status on the treatment outcome of patients with IIDDs.

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**Table 1:**

| | | AQP4 positive (n=53) | AQP4 negative (n=47) | p value |
|---|---|---|---|---|
| No. of M/ F | | 4/ 49 (1: 12.25) | 10/ 37 (1 : 3.70) | |
| Age of onset (years) | | 37.79 (±SD 13.064) | 31.74 (±SD 12.669) | 0.021* |
| Frequency of symptoms | | | | |
| | CMS | 1/ 53 (1.89%) | 27/ 47 (57.45%) | <0.001* |
| | Tumorfactive | 0/ 53 (0.00%) | 1/ 47 (2.13%) | 0.323 |
| | ON | 4/ 53 (7.55%) | 4/ 43 (8.51%) | 0.861 |
| | TM | 16/ 53 (30.19%) | 9/ 47 (19.14%) | 0.140 |
| | OS (ONTM) | 26/ 53 (49.06%) | 5/ 47 (10.64%) | <0.001* |
| | BSOS | 3/ 53 (5.65%) | 1/ 47 (2.13%) | 0.373 |
| | Cortical,OS | 1/ 53 (1.89%) | 0/ 47 (0.00%) | 0.349 |
| | BSTM | 2/ 53 (3.75%) | 0/ 47 (0.00%) | 0.159 |

M= Male, F= Female, AQP4= Aquaporin 4, SD= Standard Deviation, CMS= Classical Multiple Sclerosis, ON= Optic neuritis, TM= Transverse myelitis, ONTM= Optic neuritis and transverse myelitis, OS= Optico-spinal, BSOS= Brainstem and Optico-spinal, BSTM= Brainstem and Transverse myelitis.

**Table 2:**

| Treatment modality | Frequency (n) |
|---|---|
| 1 Treatment | 27 |
| 2 Treatments | 18 |
| 3 Treatments | 9 |
| 4 Treatments | 4 |
| 5 Treatments | 1 |

**Table 3:**

| Treatment | Total (n) | Total default (n) | AQP4 positive (n) | Treatment Failure (%) | AQP4 negative (n) | Treatment Failure (%) | P value |
|---|---|---|---|---|---|---|---|
| β Interferon | 42 | 7 | 24 | 24/ 24 (100.00%) | 11 | 2/ 11 (18.18%) | <0.005 |
| Mitoxantrone | 25 | 3 | 15 | 11/ 15 (73.33%) | 7 | 1/ 7 (14.29%) | 0.769 |
| Azathiophrine | 27 | 2 | 23 | 11/ 23 (47.83%) | 2 | 1/ 2 (50.00%) | |
| MMF | 4 | 1 | 3 | 1/ 3 (33.33%) | 0 | 0 | |
| Rituximab | 8 | 0 | 8 | 2/ 8 (25.00%) | 0 | 0 | |
| Others | 4 | 1 | 1 (Alemtuzumab) | 1/ 1 (100.00%) | 2 (Leflunamide -1, IVIG -1) | 2/ 2 (100.00%) | |

n= number, MMF= Mycophenalate myfotil, IVIG= Intravenous Immunoglobulin

**Table 4:**

| Adverse event | Frequency (%) |
|---|---|
| Leucopenia | 6/ 12 (50.00%) |
| Pancytopenia | 2/ 12 (16.67%) |
| Hepatitis | 3/ 12 (25.00%) |
| Breast carcinoma | 1/ 12 (8.33%) |

Volume : 6 | Issue : 8 | August 2016 | ISSN - 2249-555X | IF : 3.919 | IC Value : 74.50

**Table 5:**

| Treat-ment | Mean Base-line EDSS | EDSS post treat-ment | p value | Mean relapse/ year | Mean relapse/ year post treat-ment | p value |
|---|---|---|---|---|---|---|
| Azathi-ophrine | 4.27 (range 0.0-8.5) | 4.04 (range 0.0-8.5) | 0.165 | 0.89 (range 0.3 -2.9) | 0.23 (range 0-1) | 0.008* |
| Rituxi-mab | 5.92 (range 3.5-8.5) | 4.17 (range 1.0-8.5) | 0.003* | 2.10 (range 0.3-5.0) | 0.00 | 0.022* |

F= female, M= male, EDSS= Expanded disability status scale

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