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Clinical and therapeutic predictors of disease outcomes in AQP4-IgG+ neuromyelitis optica spectrum disorder

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Highlights:

- Increasing age was associated with a decreased risk of relapse
- Immunosuppression with azathioprine and mycophenolate reduced the risk of relapse
- Disability increased with age and disease duration
- Worsening of disability was slowed with rituximab, azathioprine and mycophenolate

Clinical and Therapeutic Predictors of Disease Outcomes in AQP4-IgG+ Neuromyelitis Optica Spectrum Disorder

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ABSTRACT

Background:

Aquaporin-4-IgG positive (AQP4-IgG+) Neuromyelitis Optica Spectrum Disorder (NMOSD) is an uncommon central nervous system autoimmune disorder. Disease outcomes in AQP4-IgG+NMOSD are typically measured by relapse rate and disability. Using the MSBase, a multi-centre international registry, we aimed to examine the impact immunosuppressive therapies and patient characteristics as predictors of disease outcome measures in AQP4-IgG+NMOSD.

Method: This MSBase cohort study of AQP4-IgG+NMOSD patients examined modifiers of relapse in a multivariable proportional hazards model and expanded disability status score (EDSS) using a mixed effects model.

Results: 206 AQP4-IgG+ patients were included (median follow-up 3.7 years). Age (hazard ratio [HR]=0.82 per decade, $p=0.001$), brainstem onset (HR=0.45, $p=0.009$), azathioprine (HR=0.46, $p<0.001$) and mycophenolate mofetil (HR=0.09, $p=0.012$) were associated with a reduced risk of relapse. A greater EDSS was associated with age ($\beta=0.45$ (per decade), $p<0.001$) and disease duration ($\beta=0.07$ per year, $p<0.001$). A slower increase in EDSS was associated with azathioprine ($\beta=-0.48$, $p<0.001$), mycophenolate mofetil ($\beta=-0.69$, $p=0.04$) and rituximab ($\beta=-0.35$, $p=0.024$).

Interpretation: This study has demonstrated that azathioprine and mycophenolate mofetil reduce the risk of relapses and disability progression is modified by azathioprine, mycophenolate mofetil and rituximab. Age and disease duration were

the only patient characteristics that modified the risk of relapse and disability in our cohort.

Key words: Neuromyelitis Optica Spectrum Disorder, therapy, predictors, relapses, disability, immunosuppression.

1. Introduction

Aquaporin-4-IgG positive (AQP4-IgG+) Neuromyelitis Optica Spectrum Disorder (NMOSD) is a central nervous system autoimmune disorder treated with long term immunosuppressive therapies to reduce relapses. Understanding the impact of patient characteristics and immunosuppressive therapies on disease outcomes can assist clinicians in their management decisions and prognostic stratification.

NMOSD tends to occur more often in women and has a later onset than multiple sclerosis,(1). AQP4-IgG+NMOSD is over-represented in East Asians, African-Americans and Afro-Caribbeans,(1-5). Early studies have shown that disability in NMOSD tends to accrue as a result of severe, sequential relapses with incomplete recovery,(1). Unlike relapsing-remitting multiple sclerosis, NMOSD is not considered to have a secondary progressive phase, independent of relapses,(6).

Treatment of AQP4-IgG+NMOSD involves both acute treatment for relapses and long term immunosuppression with therapies including; rituximab,(7), mycophenolate mofetil,(8), azathioprine,(9) and methotrexate,(10). Newer therapies with efficacy include; eculizumab,(11), tocilizumab,(12) and inebilizumab,(13). Disease modifying therapies directed multiple sclerosis have been reported to worsen outcomes in AQP4-IgG+NMOSD, (14,15,16).

The impact of patient characteristics and immunosuppression upon relapse in AQP4-IgG+NMOSD have been studied mainly in isolation (either patient characteristics or immunosuppression separately) and there is limited information about long term disability associated with AQP4-IgG+NMOSD. There is a need to study patient characteristics and immunosuppressive therapies together in large, multi-centre international cohorts, outside of clinical trials, to provide a greater depth of understanding of these predictors upon outcomes. Therefore, using the MSBase registry, we aimed to investigate the impact of immunosuppressive therapies and patient characteristics on relapse rate in AQP4-IgG+NMOSD. We aimed to additionally evaluate the impact of these upon disability outcomes in AQP4-IgG+NMOSD.

2. Methods

2.1 Ethics

MSBase,(18) (registered with WHO ICTRP, ID ACTRN12605000455662) was approved by the Melbourne Health Human Research Ethics Committee, and by the local ethics committees in all participating centres (or exemptions granted, according to applicable local laws and regulations). If required, written informed consent was obtained from enrolled patients, in accordance with the Declaration of Helsinki. A standardised data quality process was applied to the used data set,(19).

2.2 Study population

The study included patients from the MSBase cohort (NMOBase substudy) who fulfilled the following criteria: clinical diagnosis of AQP4-IgG+NMOSD as per Wingerchuk 2015,(17) diagnostic criteria (including retrospective diagnoses), availability of the minimum dataset (MS centre details, patient year of birth, sex, date of disease onset, clinical relapses and at least 2 clinic visits with recorded expanded disability status scores (EDSS)). The majority of patients were tested for AQP4-IgG using cell based assays (Euroimmun, Mayo Clinic fluorescence-activated cell sorting assay or in-house cell based assay). Other techniques used by a minority of centres included immunofluorescence (patients, n=29) and ELISA (patients, n=9).

The data was recorded mostly at large tertiary centres at the time of clinical visits. The data entry portal was either the iMed patient record system or the MSBase online data entry system.

2.3 Study design

This is a retrospective cohort study. The study period was the time between the first and last recorded EDSS visits. The analyses examined associations of patient characteristics and immunosuppressive therapies with the risk of relapses and change in EDSS.

2.4 Modifiers of disease outcome

The patient characteristics included; age, disease duration, sex, Asian ethnicity, anatomical location of first symptom (optic pathways, spinal cord, brainstem and/or supratentorial) and proportion of time pregnant (for pregnancies which occurred after disease onset).

The immunosuppressive therapies included: autologous stem cell transplant, azathioprine, cyclosporin, cyclophosphamide, dimethyl fumarate, eculizumab, fingolimod, glatiramer acetate, hydroxychloroquine, interferon- β , methotrexate, mitoxantrone, mycophenolate mofetil, natalizumab, rituximab, tocilizumab. Exposure to acute therapies for relapses, corticosteroids, intravenous immunoglobulin (IVIG), plasma exchange, were treated as nuisance variables.

Upon cessation of a therapy, the durations of treatment effect were estimated as per Stellmann et al,(20); 180 days for rituximab; 90 days for mitoxantrone, 30 days for azathioprine, cyclosporin, mycophenolate mofetil, natalizumab, IVIG, fingolimod, intravenous or oral corticosteroids, tocilizumab and 7 days for interferon- β , glatiramer acetate, methotrexate and long term corticosteroids (oral). The duration of effect of other therapies was based on clinical experience and mechanism of action; the effect of cyclophosphamide was estimated to be 90 days, plasma exchange and tocilizumab were estimated to be 30 days, eculizumab was estimated to be 180 days. The treatment effect of autologous stem cell transplant was estimated as 5 years,(15).

2.5 Disease outcomes

Two study endpoints were examined: cumulative hazard of clinical relapses and EDSS trajectories. A relapse was defined as occurrence of new symptoms or exacerbation of existing symptoms persisting for at least 24-hours, in the absence of concurrent illness or fever,(21). Relapses which occurred within 30 days of a previous relapse were not included in the analysis. EDSS was recorded at each centre by neurostatus certified EDSS raters, as stipulated by the MSBase Observational Plan,(22).

2.6 Statistical analysis

Statistical analyses were carried out using R version 3.4.3. Two statistical analyses were completed for predictors of relapse and predictors of disability. The associations between patient characteristics and immunosuppressive therapies and the cumulative hazard of relapses were examined using Andersen-Gill proportional hazards model. Variables in the model included; age, disease duration, sex, pregnancy, site of disease onset, Asian ethnicity, immunosuppressant therapies. Treatment was modeled as a time-dependent variable, allowing for a combination of therapies where the epochs of expected treatment effectiveness overlapped. Only the treatment time recorded between the first and last visit was analysed. All immunosuppressant therapies were included in the analysis, however, therapies with less than 15 treatment episodes are not reported. Pregnancy and exposure to corticosteroids were quantified as the proportions of time between the first and the last recorded EDSS score that a patient was pregnant or treated with corticosteroids.

The associations between the patient characteristics and immunosuppressive therapies and disability trajectories were studied with a mixed effects regression model with random intercept per patient ID (cluster variable). The same variables were included in this mixed effects model as were included in the Anderson-Gill model for relapses, apart from baseline EDSS.

A sensitivity analysis was conducted using the same Anderson-Gill model for relapses, without the patients who had only received one rituximab treatment episode less than 180 days in their disease course.

3. Results

3.1 Study cohort

705 NMOSD patients in the MSBase cohort, recruited between 1979 and 2017 were assessed for eligibility (including patients who were retrospectively diagnosed as NMOSD). Of these, 495 NMOSD patients had a minimum of 2 clinic visits with EDSS recorded. There were 206 patients who met the 2015 International criteria for AQP4-IgG+NMOSD and were included. The 289 patients were excluded due to seronegative serostatus or the serostatus information was incomplete. The patients' mean age at first visit was 43 years, with 91% of patients being female and median disease duration at the final visit of 7.1 years (Table 1).

3.2 Patient characteristics and association with relapse rate

Relapses tended to be most common during the fourth decade of life, with no apparent association with the time from first symptom (Figure 1A and B). Age (per decade) (HR=0.82, $p=0.001$) and brainstem site of disease onset (HR=0.45, $p=0.009$) were associated with a lower risk of relapse (Figure 2). Asian ethnicity, female sex, pregnancy, supratentorial, optic nerve or spinal cord onset were not predictive of relapses.

3.3 Immunosuppressive therapies and association with relapse rate

All exposures to immunosuppressive therapies were included in the study. The majority of the patients were treated with azathioprine (patients, $n=122$, treatment episodes, $n=136$). Treatment with azathioprine (HR=0.46, $p<0.001$) and mycophenolate mofetil (HR=0.09, $p=0.011$) were associated with a reduced risk of relapse (Figure 2). There were 84 patients treatments with 169 treatment episodes of rituximab. Exposure to rituximab was trending towards an association with lower risk of relapse but did not reach statistical significance (HR=0.73, $p=0.17$). Sensitivity analyses with the 11 patients whom had rituximab treatment epochs shorter than 180 days removed revealed no significant change in the coefficients (Supplementary table e4).

3.4 Patient characteristics and association with disability

As shown in Figure 1C, disability tended to increase over time. Age ($\beta=0.45$ per decade, $p<0.001$) and disease duration ($\beta=0.07$ per year, $p<0.001$) were associated

with a greater EDSS (Figure 3). Asian ethnicity, pregnancy, site of disease onset did not impact disability outcomes.

3.5 Immunosuppressive therapies and association with disability

Treatment with azathioprine ($\beta=-0.48$, $p<0.001$), mycophenolate mofetil ($\beta=-0.69$, $p=0.04$) and rituximab ($\beta=-0.35$, $p=0.024$), were associated with slower increase in EDSS (Figure 3). Cyclophosphamide ($\beta=1.1$, $p=0.041$), intravenous immunoglobulin ($\beta= 2.03$, $p<0.001$) and plasma exchange ($\beta=1.33$, $p<0.001$) were associated with a greater increase in disability (Figure 3).

4. Discussion

This study examines the impact of patient characteristics and immunosuppressive therapies upon clinical outcomes throughout the relapse rate and disability in an international, cohort of 206 AQP4-IgG+NMOSD patients with a median follow up time of 3.7 years.

We have demonstrated that standard immunosuppressive therapies commonly used in AQP4-IgG+NMOSD (azathioprine, mycophenolate mofetil and rituximab) tend to be associated with lower disability and reduced risk of relapses. The incidence of relapses declines with age and the disability increases over time (whether expressed as age or disease duration). Brainstem onset of disease was associated lower relapse activity.

Immunosuppression is the mainstay of therapy for AQP4-IgG+NMOSD. Several cohort studies have examined frequency of relapses and disability before and after commencement with a single immunosuppressive therapy. In this study, we evaluated outcomes of treatment with a number of immunosuppressive therapies, while accounting for patient characteristics We found evidence for reduced relapse rate and slower disability accrual in patients with AQP4-IgG+NMOSD treated with azathioprine and mycophenolate mofetil. In agreement with our study, other studies have demonstrated a decrease in relapse frequency and improved disability outcomes after patients commenced mycophenolate mofetil,(8, 23) and azathioprine,(9, 24-26).

Rituximab is typically used as a second-line therapy in patients with suboptimal response to first-line immunosuppressive agents. It has been reported to reduce relapse frequency in several studies,(7). In this study we found a strong trend towards lower incidence of relapses on rituximab, but this did not reach the formal level of statistical significance. Whether a trend reaches statistical significance in the frequentist framework is the function of magnitude and variability of the studied association. Hence, the lack of the statistically confirmed association between rituximab and lower relapse frequency speaks about the variability of the observations in our two studied samples. This could be potentially explained by the variable context in which this second-line therapy is used. Further, response to rituximab amongst AQP4-IgG+NMOSD patients may not be uniform and as demonstrated in patients with rheumatoid arthritis, and there may be genetic variability in response to rituximab,(27).

Cyclophosphamide, intravenous immunoglobulin and plasma exchange are more likely to be used in severe NMOSD relapses; therefore, their association with increasing disability most likely represents a reverse causation that is secondary to indication bias. However, cyclophosphamide has been previously reported to lack efficacy in a small cohort of 7 AQP4-IgG+NMOSD patients,(28).

In our evaluation of patient characteristics we found that age represents an important determinant of the frequency of episodic inflammatory events in AQP4-IgG+NMOSD. Similar to a German cohort, we found that for every decade of age, the risk of relapses decreased (20). Time from the first symptom onset of AQP4-IgG+NMOSD was not associated with the risk of relapse. The majority of studies in AQP4-IgG+NMOSD focus on relapse rate and there are limited studies examining disability thus far. In this study we found that increasing disability was associated with increasing age and disease duration. This association is expected, as disability in AQP4-IgG+NMOSD typically results from accumulation of disability through episodic exacerbations of the disease. These observations suggest that long-term immunosuppression is most likely a necessity in treatment of AQP4-IgG+NMOSD, even if the frequency of relapses may decline in older patients.

An early study of a NMOSD (seropositive and seronegative) cohort found that female sex was associated with a relapsing course in NMOSD prior to AQP4-IgG testing,(29). We have not observed an effect of sex in this study, however only 9% of our AQP4-IgG+NMOSD cohort were male.

Previous studies reported increased risk of relapses in NMOSD in association with pregnancy. This increase was seen mainly during the first 3 months of post-partum period,(30, 31). We captured 21 pregnancies within the study period. The majority of patients were untreated during pregnancy; however, 2 patients were treated with rituximab, 3 were partially treated (for <20% of pregnancy) with azathioprine, and 1 patient was treated with glatiramer acetate. We did not find any associations between pregnancy and relapse activity or disability.

The effect of ethnicity on incidence and disease severity is not well understood, however, a North American multi-centre study of AQP4-IgG+NMOSD of both seropositive and seronegative patients found that patients of African descent were overrepresented in their cohort,(3). Furthermore, AQP4-IgG+NMOSD patients with African ancestry have been reported to have a higher rate of mortality in comparison to a pooled cohort of AQP4-IgG+NMOSD,(32). A cohort study reported that AQP4-IgG+NMOSD patients from the United Kingdom (UK) (n=59) had more severe disease, more severe onset attacks, higher relapse frequency and greater disability at follow up in comparison to a similar cohort from Japan (n=47),(2). Within this UK cohort, Afro-Caribbeans had a higher probability of visual disability and Caucasians were more likely than Asians to develop motor disability or wheelchair dependence,(2). In Australia, the prevalence of AQP4-IgG+NMOSD has been reported to be higher among Asian population than Caucasian population,(33). A multi-centre study of clinical predictors in NMOSD recently demonstrated that Japanese patients had lower risk of relapses compared to Caucasians but paradoxically had a highest brainstem attack risk,(34). Another multi-centre study found that severe attacks at onset occurred more frequently in Afro-American/Afro-European patients than Asian or Caucasian patients (35). We did not find an effect of Asian ethnicity on relapse or disability. Our study was not powered to analyse the association with African ethnicity. In the future, prognostication of AQP4-IgG+NMOSD may be further elucidated with genome-wide association studies.

We observed that brainstem onset of disease (compared to non-brainstem onset) was associated with an overall lower relapse frequency. We postulate that brainstem onset of disease may represent patients with predominantly area postrema syndrome and that therefore may represent a more benign disease subgroup. We did not find a significant effect for optic nerve, spinal cord or supratentorial onset on the risk of relapse or disability in this cohort.

The main strength of this study consists in the size of this longitudinally followed international AQP4-IgG+NMOSD cohort, drawn from 40 MSBase centres from 19 countries with a median follow up of 3.7 years and the analysis of multiple clinical determinants and immunosuppressive therapies on relapses and disability together in well powered multivariable models. Such approach is critical for objective evaluation of the effectiveness of the current AQP4-IgG+NMOSD treatment strategies, as non-interventional factors, such as patient age, disease onset and pregnancy, represent modifiers of AQP4-IgG+NMOSD outcomes.

The limitations of this study are inherent in the observational nature of the analysed data. To maximise reliability of the analysed data, we have used an objective data quality procedure. This study evaluated effectiveness of therapies for AQP4-IgG+NMOSD as they were utilized by their treating neurologists. While this was a source of variability in utilization and dosing of therapies, this approach enabled us to establish associations of the current practice of treating AQP4-IgG+NMOSD. This study was not sufficiently powered to enable comparisons of the effectiveness between different therapies. While the multivariable models were adjusted for multiple presumed confounders of treatment allocation and disease outcome, such adjustment is subject to residual indication bias, especially where the cohort size is limited. Further studies in well powered groups using statistical methodology to minimize treatment indication bias will allow comparison between therapies.

Current use of broad immunosuppressants and B cell therapy in management of AQP4-IgG+NMOSD reduce the risk of relapses and disability progression. Age and disease duration were the only patient characteristics that modified the risk of relapse

and disability in our cohort. Further head-to-head comparisons of individual therapies in properly adjusted large cohorts is needed.

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Ilya Kister served on scientific advisory board for Biogen and received research support from Guthy-Jackson Charitable Foundation, National Multiple Sclerosis Society, Biogen and Novartis.

Tomas Kalincik served on scientific advisory boards for Roche, Genzyme-Sanofi, Novartis, Merck and Biogen, steering committee for Brain Atrophy Initiative by Genzyme, received conference travel support and/or speaker honoraria from WebMD Global, Novartis, Biogen, Genzyme-Sanofi, Teva, BioCSL and Merck and received research support from Biogen.

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Figure legends

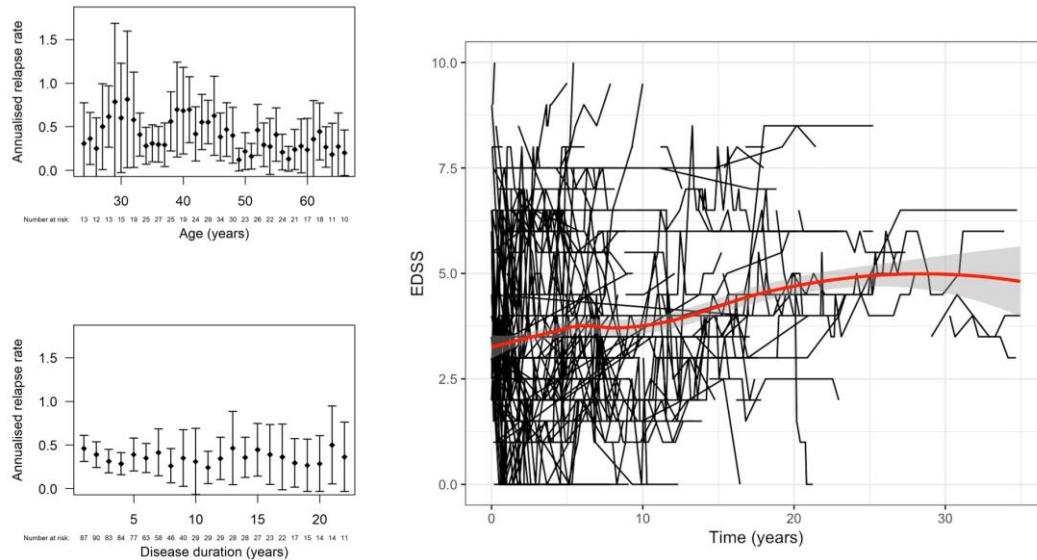


Figure 1: ARR with respect to age (A) and disease duration (B), EDSS over time (C) in AQP4-IgG-positive NMOSD

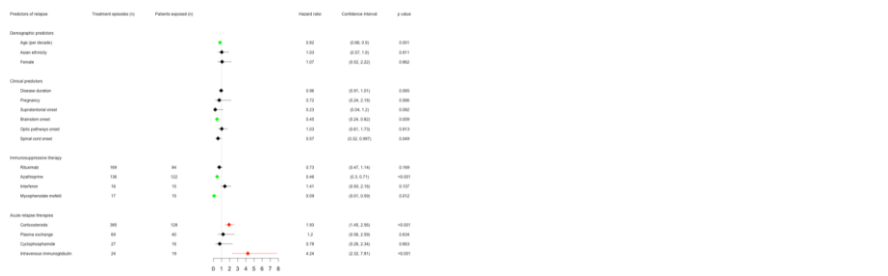


Figure 2: Forest plot of the clinical and therapeutic predictors of relapse in AQP4-IgG-positive NMOSD

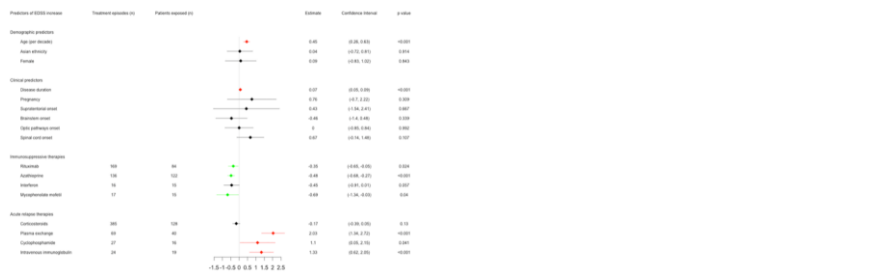


Figure 3: Forest plot of the clinical and therapeutic predictors of disability in AQP4-IgG-positive NMOSD

Table 1: Baseline Characteristics of AQP4-IgG+ NMOSD cohort

| Patient Characteristics | AQP4-IgG positive NMOSD n=206 |
|--|--|
| Demographics | |
| Females, n (%) | 188 (91%) |
| Age at first visit, mean (SD), years | 43 (15) |
| Non-Asian ethnicity, n (%) | 148 (72%) |
| Asian ethnicity, n (%) | 31 (15%) |
| Ethnicity unspecified, n (%) | 27 (13%) |
| Clinical features | |
| Follow up time, median, years | 3.7 (2.7-4.7) |
| Disease duration at final visit, median, years | 7.1 (5.1-9.1) |
| ARR at final visit, mean | 0.35 |
| Baseline EDSS, median (IQR) | 3 (2.5-3.5) |
| Initial NMOSD presentation, n | |
| Brainstem | 23 |
| Optic pathways | 104 |
| Supratentorial | 4 |
| Spinal cord | 96 |
| AQP4-IgG positive, n | 206 |
| Pregnancies within study period, n | 21 |
| Immunosuppressive therapies | Treatment episodes, n (patients exposed, n) |
| Azathioprine | 136 (122) |
| Rituximab | 169 (84) |
| Interferon beta | 16 (15) |
| Mycophenolate mofetil | 17 (15) |
| Glatiramer acetate | 5 (5) |
| Tocilizumab | 7 (6) |
| Ecilizumab | 5 (5) |
| Methotrexate | 4 (4) |
| Mitoxantrone | 3 (3) |
| Natalizumab | 3 (2) |
| Hydroxychloroquine | 3 (2) |
| Autologous stem cell transplant | 2 (2) |
| Cyclosporin | 1 (1) |
| Acute relapse therapies | |
| Corticosteroids | 385 (128) |
| Plasma exchange | 69 (40) |
| Cyclophosphamide | 27 (16) |
| Intravenous Immunoglobulin | 24 (19) |

Abbreviations: n= number, SD=standard deviation, IQR = interquartile range