**Immunotherapies in neuromyelitis optica spectrum disorder: efficacy and predictors of response**

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**ABSTRACT**

**Objective** To analyse predictors for relapses and number of attacks under different immunotherapies in patients with neuromyelitis optica spectrum disorder (NMOSD).

**Design** This is a retrospective cohort study conducted in neurology departments at 21 regional and university hospitals in Germany. Eligible participants were patients with aquaporin-4-antibody-positive or aquaporin-4-antibody-negative NMOSD. Main outcome measures were HRs from Cox proportional hazard regression models adjusted for centre effects, important prognostic factors and repeated treatment episodes.

**Results** 265 treatment episodes with a mean duration of 442 days (total of 321 treatment years) in 144 patients (mean age at first attack: 40.9 years, 82.6% female, 86.1% aquaporin-4-antibody-positive) were analysed. 191 attacks occurred during any of the treatments (annual relapse rate=0.60). The most common treatments were rituximab (n=77, 111 patient-years), azathioprine (n=52, 68 patient-years), interferon-β (n=32, 61 patient-years), mitoxantrone (n=34, 32.1 patient-years) and glatiramer acetate (n=17, 10 patient-years). Azathioprine (HR=0.4, 95% CI 0.3 to 0.7, p=0.001) and rituximab (HR=0.6, 95% CI 0.4 to 1.0, p=0.034) reduced the attack risk compared with interferon-β, whereas mitoxantrone and glatiramer acetate did not. Patients who were aquaporin-4-antibody-positive had a higher risk of attacks (HR=2.5, 95% CI 1.3 to 5.1, p=0.009). Every decade of age was associated with a lower risk for attacks (HR=0.8, 95% CI 0.7 to 1.0, p=0.039). A previous attack under the same treatment tended to be predictive for further attacks (HR=1.5, 95% CI 1.0 to 2.4, p=0.065).

**Conclusions** Age, antibody status and possibly previous attacks predict further attacks in patients treated for NMOSD. Azathioprine and rituximab are superior to interferon-β.
German Neuromyelitis Optica Study group (NEMOS), we analysed the efficacy of immunotherapies for attack prevention and predictors for attacks under immunotherapies.

METHODS
Study design and patients
This retrospective cohort study was based on the German NEMOS group (www.nemos-net.de) registry established in 2008. At database lock, the registry included 186 patients with neuromyelitis optica (NMO) diagnosed according to the 2006 Wingerchuk criteria or with AQP4-Ab-positive NMO spectrum disorder (NMOSD). The local institutional review boards of the participating centres approved the study (first approval from the institutional review board Charité Universitätsmedizin Berlin EA3/004/08). Last data entry for this analysis varied between centres and was between January 2012 and March 2013.

Data collection and processing
Data collected at regular clinical visits included demographic data, AQP4-Ab status, attacks (onset, treatments and outcome), long-term treatments (compounds, start/stop dates and dosages), expanded disability status scale and visual acuity. A detailed description of the cohort and the methods used for data collection, including an on-site data validation (‘flying doctor-approach’), has been published previously.8

For this study, demographic data, long-term treatment data and attack dates were extracted from the database. Treatment data were validated through manual quality checks performed by two authors (JPS and MK), as well as by automated logical checks. As further analyses relied on exact treatment data, including start and stop dates, patients with insufficient baseline or treatment data were excluded.

Definition of treatment episodes
For our analyses which were based on pharmacodynamics and previous treatment experience, we determined the efficacy of therapeutic interventions after the last dose as follows: 365 days for alemtuzumab; 180 days for RTX; 90 days for mitoxantrone (Mitox) and intrathecal steroids; 30 days for cyclophosphamide, AZA, ciclosporin A, MMF, natalizumab, intravenous immunoglobulin, fingolimod (FTY), intravenous steroids and tocilizumab (TCZ); and 7 days for interferon-β (IFN), glatiramer acetate (GLAT), methotrexate and oral steroids. Treatment duration was prolonged if the documented stop date (ie, clinical decision to not further proceed with the current treatment) was before the end of effectiveness as defined above. In recurrent treatment episodes with the same compound (observed for AZA, RTX, Mitox and IFN), we merged the two cycles if the first dose of the second episode was administered less than 30 days (AZA, RTX, Mitox) or 10 days (IFN) after the assumed end of effectiveness, for example two RTX cycles 200 days apart were considered as one continuous treatment episode.

For other treatments, no recurrent treatment episodes were observed. Gaps longer than the mentioned period were treated as separated episodes in further analyses. Treatments with uncertain treatment start or stop dates were excluded. Finally, in 59 treatments an overlap of treatment durations as defined by the above-mentioned effectiveness time or a combination of two or three compounds occurred (online supplementary table I). Unfortunately, these groups were too small and too heterogeneous to reliably investigate combination therapies. Therefore, our analyses were restricted to monotherapies.

Data sets
Two data sets were defined. Data set A included all available treatment data for descriptive statistics, its changes over time and for computing unadjusted annual relapse rates (ARR).

In Data set B, recurrent event analyses considering the time from treatment start to attack were performed. Treatment episodes from patients with an attack under the treatment were split in separate episodes by the date of attack onset. Each treatment episode was labelled as stable if no attack occurred and as failure if an attack occurred. Treatment episodes shorter than 14 days or with unreliable start and stop dates were excluded. For efficacy analyses and response predictors, we reduced the data set to compounds with at least 10 patient-years in at least 10 patients (Data set B).

Statistical analyses
Our statistical analysis plan was designed (1) to investigate prescription reality of immunotherapies in Germany and their changes over time, (2) to compare efficacy of treatments in recurrent event analyses and (3) to explore the data set for predictors of relapses.

Using Data set A, descriptive statistics of the cohort were performed. To investigate if prescription routine changed over time, the χ2 test was used to compare frequencies before and after publication of the German NMO treatment guidelines by NEMOS in early 2011.26 Under the assumption that attack occurrence follows a Poisson distribution, we estimated annualised relapse rates (ARR, mean number of relapses per treatment year) and 95% CIs. ARR estimates were unadjusted for any covariates. Differences between the cohorts were tested with χ2 test (rates) or analysis of variance (continuous data).

For the analysis of Data set B, the multivariate Cox proportional hazard regression models for recurrent events were computed.27 We aimed to compare treatment effects and to investigate the influence of potential predictors of attack risk: age, gender, previous attack under therapy, line of treatment for the individual patient (labelled as first line, second line, or third or more line), AQP4-Ab status and whether the 2006 Wingerchuk criteria were fulfilled. The patient ID was included as cluster variable to account for intrindividual correlation of observations, and the models were additionally corrected for centre effects. In a first step, a multivariate model was performed, including predictors and treatments, and HRs and their 95% CIs were computed. Second, the number of variables in the model was reduced by excluding all variables not showing at least a trend towards significance (defined as p<0.1). As no untreated or placebo cohort for estimating HRs was available, IFN was chosen as a reference category for all treatment comparisons, as IFN was shown to be without clinical efficacy in NMOSD.28 To compare predictors between different treatments, we performed post-hoc analyses for each treatment subgroup. p Values <0.05 were considered statistically significant. All analyses were performed with Statistics in R (V3.2.3), including the survival package.29

RESULTS
Description of the cohort
One hundred and eighty-six patients with NMO or AQP4-Ab-positive NMOSD were documented in the NEMOS registry. After exclusion of incomplete and uncertain treatment data as well as overlapping treatments, 265 different treatments in 144 patients from 21 centres could be extracted (figure 1). The majority of patients were female (n=119, 82.6%), fulfilled the 2006 Wingerchuk diagnostic criteria (n=113, 78.5%) and were seropositive...
for AQP4-ab (n=124, 86.1%). The mean age at disease onset was 40.9 (SD: 14.3) years and the median follow-up time was 6.1 (0.1–34.9) years. The median number of immunotherapies per patient was 2 (1–8). The mean duration of treatments was 442 (SD: 432) days, summing up to a total of 321 documented treatments. One hundred and thirty-seven treatments were given as first-line therapy in the individual patient, 69 as second-line and 59 as third-line (or more). One hundred and ninety-one attacks occurred during the documented treatments, resulting in an overall ARR of 0.60 (95% CI 0.51 to 0.69) in treated patients.

Frequencies, prescription changes and unadjusted attack rates for all treatments
We identified 15 different immunotherapy drugs, which were used in 1–65 (median: 7) patients (table 1). The most commonly used compounds were RTX, with 111 patient years in 65 patients, AZA with 68 patient-years in 46 patients and IFN with 61 patient-years in 30 patients. The mean treatment duration ranged from 77 (FTY) to 699 (IFN) days. Unadjusted ARRs and 95% CI of all treatments are presented in table 1. The prescription of compounds changed over time. Of the most commonly prescribed drugs, RTX (first given in 2005) showed an increase from 24% to 43% of all prescriptions with the cut-off of 2011, whereas Mitox use decreased from 16% to 2% and IFN treatment was not further initiated after 2011. The use of all medications over time and of all relapses occurring during treatment are visualised in figure 2.

Detailed dosing data for these treatments were available in most cases (AZA: 94%, GLAT: 78%, IFN: 88%, Mitox: 87%, RTX: 98%). GLAT patients received 20 mg/day, 69% of IFN treatments used the full dose and 31% a reduced dose (eight times IFN-β-1-a subcutaneous 22 µg, one time IFN-β-1-a intramuscular 8 mg). The median dose of Mitox was 12 mg/m² body surface (range: 10–12 mg) every 3 months. The median dose of AZA was 150 mg per day (mean: 126 mg, range: 50–300 mg). Fifty per cent of AZA doses were 150 mg or above and 82% at least 100 mg/day. However, sufficiency of AZA dosing could not be reliably evaluated, as body weight data were not available. The median dose of 1000 mg per cycle (one or two infusions) of RTX (range: 375–3000 mg) or more was applied in 79% of treatments, usually every 6 months.
Table 1 Descriptive statistics per treatment (data set A)

<table>
<thead>
<tr>
<th>AZA</th>
<th>CsA</th>
<th>CYC</th>
<th>FTY</th>
<th>GLAT</th>
<th>IFN</th>
<th>IVIG</th>
<th>Mitox</th>
<th>MMF</th>
<th>MTX</th>
<th>NAT</th>
<th>RTX</th>
<th>Steroids i.th.</th>
<th>Steroids p.o.</th>
<th>TCZ</th>
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<tbody>
<tr>
<td>46</td>
<td>1</td>
<td>12</td>
<td>2</td>
<td>17</td>
<td>30</td>
<td>3</td>
<td>34</td>
<td>8</td>
<td>5</td>
<td>7</td>
<td>65</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>42 (91.3)</td>
<td>1 (100)</td>
<td>12 (100)</td>
<td>2 (100)</td>
<td>16 (94.1)</td>
<td>25 (83.3)</td>
<td>3 (100)</td>
<td>26 (76.5)</td>
<td>5 (62.5)</td>
<td>5 (100)</td>
<td>5 (71.4)</td>
<td>54 (83.1)</td>
<td>4 (80.0)</td>
<td>4 (80.0)</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>43.8 (12.6)</td>
<td>43.5 (8.5)</td>
<td>47 (11.9)</td>
<td>54.3 (3.4)</td>
<td>40.5 (9.6)</td>
<td>37.6 (11.4)</td>
<td>41.1 (22.6)</td>
<td>46.8 (13.4)</td>
<td>53.2 (12.0)</td>
<td>50.8 (11.9)</td>
<td>48.4 (10.7)</td>
<td>46.8 (12.8)</td>
<td>46.2 (9)</td>
<td>43.2 (16.4)</td>
</tr>
<tr>
<td>Disease duration, mean (SD), years</td>
<td>4.4 (6)</td>
<td>13.4 (8.5)</td>
<td>4.2 (5.9)</td>
<td>18.6 (1.4)</td>
<td>6.1 (5.8)</td>
<td>5.7 (6.2)</td>
<td>7.8 (7.9)</td>
<td>5.4 (5.9)</td>
<td>5.6 (8.0)</td>
<td>5.6 (8.3)</td>
<td>11.6 (9.1)</td>
<td>4.8 (4.8)</td>
<td>4.9 (5.1)</td>
<td>2.7 (3.8)</td>
</tr>
<tr>
<td>2006 Wingerchuk criteria fulfilled, n (%)</td>
<td>33 (71.7)</td>
<td>1 (100)</td>
<td>10 (83.3)</td>
<td>2 (100)</td>
<td>14 (83.4)</td>
<td>29 (96.7)</td>
<td>2 (66.7)</td>
<td>27 (79.4)</td>
<td>6 (75.0)</td>
<td>4 (80.0)</td>
<td>7 (100)</td>
<td>50 (76.9)</td>
<td>4 (80.0)</td>
<td>4 (80.0)</td>
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<tr>
<td>AQP4-ab-positive, n (%)</td>
<td>40 (87.0)</td>
<td>1 (100)</td>
<td>12 (100)</td>
<td>1 (50)</td>
<td>11 (64.7)</td>
<td>27 (90.0)</td>
<td>3 (100)</td>
<td>30 (88.2)</td>
<td>7 (87.5)</td>
<td>4 (80.0)</td>
<td>7 (100)</td>
<td>57 (87.7)</td>
<td>4 (80.0)</td>
<td>4 (80.0)</td>
</tr>
<tr>
<td>Treatment episodes n (%)</td>
<td>52 (19.6)</td>
<td>2 (0.8)</td>
<td>14 (5.3)</td>
<td>2 (0.6)</td>
<td>17 (6.4)</td>
<td>32 (12.1)</td>
<td>3 (0.9)</td>
<td>34 (12.8)</td>
<td>8 (3.0)</td>
<td>5 (1.9)</td>
<td>7 (2.1)</td>
<td>77 (29.1)</td>
<td>5 (1.9)</td>
<td>5 (1.9)</td>
</tr>
<tr>
<td>Episodes without attack, %</td>
<td>67.3</td>
<td>50</td>
<td>71.4</td>
<td>50</td>
<td>52.9</td>
<td>25.0</td>
<td>33.3</td>
<td>64.7</td>
<td>37.5</td>
<td>60.0</td>
<td>28.6</td>
<td>59.7</td>
<td>80.0</td>
<td>100</td>
</tr>
<tr>
<td>First-line, n</td>
<td>31</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>7</td>
<td>27</td>
<td>2</td>
<td>22</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>28</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Second-line, n</td>
<td>10</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>0</td>
<td>9</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td>24</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Third-line or more, n</td>
<td>11</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
<td>25</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rate of first-line treatments per drug, %</td>
<td>59.6</td>
<td>0</td>
<td>64.3</td>
<td>0</td>
<td>41.2</td>
<td>84.4</td>
<td>66.7</td>
<td>64.7</td>
<td>37.5</td>
<td>20.0</td>
<td>28.6</td>
<td>36.4</td>
<td>20.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Rate of drug as first-line in the cohort, %</td>
<td>22.6</td>
<td>0</td>
<td>6.6</td>
<td>0</td>
<td>5.1</td>
<td>19.7</td>
<td>1.5</td>
<td>16.1</td>
<td>2.2</td>
<td>0.7</td>
<td>1.5</td>
<td>20.4</td>
<td>0.7</td>
<td>2.9</td>
</tr>
<tr>
<td>Treatment duration, mean (SD) days</td>
<td>475 (477)</td>
<td>291 (281)</td>
<td>122 (125)</td>
<td>77 (21)</td>
<td>221 (186)</td>
<td>699 (596)</td>
<td>488 (331)</td>
<td>345 (268)</td>
<td>462 (447)</td>
<td>363 (335)</td>
<td>159 (104)</td>
<td>524 (430)</td>
<td>418 (309)</td>
<td>165 (186)</td>
</tr>
<tr>
<td>Cumulative treatment, years</td>
<td>67.6</td>
<td>1.6</td>
<td>4.7</td>
<td>0.4</td>
<td>10.3</td>
<td>61.2</td>
<td>4.0</td>
<td>32.1</td>
<td>10.1</td>
<td>5.0</td>
<td>3.0</td>
<td>110.5</td>
<td>5.7</td>
<td>2.3</td>
</tr>
<tr>
<td>Annualised attack rate, mean (95% CI)</td>
<td>0.34 (0.22–0.51)</td>
<td>0.63 (0.02 to 3.48)</td>
<td>1.28 (0.47 to 3.02)</td>
<td>2.37 (0.06 to 13.02)</td>
<td>1.36 (0.74 to 2.28)</td>
<td>0.75 (0.55 to 1.00)</td>
<td>0.75 (0.15 to 2.18)</td>
<td>0.84 (0.55 to 1.55)</td>
<td>0.79 (0.34 to 1.55)</td>
<td>0.40 (0.05–1.45)</td>
<td>1.97 (0.73 to 4.28)</td>
<td>0.46 (0.34 to 0.61)</td>
<td>0.35 (0.04 to 1.26)</td>
<td>0 (0. to 1.63)</td>
</tr>
</tbody>
</table>

Age and disease duration at treatment start:
AZA, azathioprine; AQP4-ab, antibodies against aquaporin-4; CsA, ciclosporin A; CYC, cyclophosphamide; FTY, fingolimod; GLAT, glatiramer acetate; IFN, interferon-β; IVIG, intravenous immunoglobulins; i.th., intrathecal; Mitox, mitoxantrone; MMF, mycophenolate mofetil; MTX, methotrexate; NAT, natalizumab; p.o., per os; RTX, rituximab; TCZ, tocilizumab.
Neuro-inflammation

The compounds AZA, IFN and RTX were the most often used first-line therapies in our cohort. RTX was the most often used second-line or third-line therapy. The sequence of compounds used was very heterogeneous, and we did not observe any obvious pattern of typical therapy sequences.

Analysis of predictors of attack risk

Five treatments (AZA, GLAT, IFN, Mitox and RTX) had at least 10 patient-years in at least 10 patients. Three hundred and twenty-two treatment episodes from 127 patients (20 centres) labelled as ‘stable’ (n=191, 59%) or ‘failure’ (n=131, 41%) were included in these analyses. The observation time in this data set included a total of 261.7 patient-years. The five treatment cohorts did not differ significantly concerning gender, proportion of patients positive for AQP4-ab or meeting the 2006 Wingerchuk criteria,25 respectively (table 2).

Differences between groups tested with χ² test for rates or analysis of variance for continuous measures.

AQ4-ab, antibodies against aquaporin-4; AZA, azathioprine; GLAT, glatiramer acetate; IFN, interferon-β; Mitox, mitoxantrone; NA, not applicable; RTX, rituximab.
Neuro-inflammation

Figure 3  HRs for predictors and treatments. HR and 95% CIs for clinical predictors of treatment response and different treatments (interferon-β as reference). Age and disease duration as decades. Variables not included in the final model: line of therapy, gender and whether the 2006 Wingerchuk criteria were fulfilled. For details, see the Methods section. AQP4-ab, antibodies against aquaporin-4; AZA, azathioprine; GLAT, glatiramer acetate; Mitox, mitoxantrone; RTX, rituximab. * Represent statistically significant data.

<table>
<thead>
<tr>
<th>Predictors for attacks</th>
<th>HR [95% CI]</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AQP4-ab positive</td>
<td>2.5 [1.3-5.1]</td>
<td>0.009*</td>
</tr>
<tr>
<td>GLAT</td>
<td>1.5 [0.8-2.6]</td>
<td>0.188</td>
</tr>
<tr>
<td>Previous attack under same therapy</td>
<td>1.5 [1-2.4]</td>
<td>0.065</td>
</tr>
<tr>
<td>Mitox</td>
<td>0.9 [0.5-1.6]</td>
<td>0.639</td>
</tr>
<tr>
<td>Disease duration decades</td>
<td>0.8 [0.6-1]</td>
<td>0.107</td>
</tr>
<tr>
<td>Age decades</td>
<td>0.8 [0.7-1]</td>
<td>0.039*</td>
</tr>
<tr>
<td>RTX</td>
<td>0.6 [0.4-1]</td>
<td>0.034*</td>
</tr>
<tr>
<td>AZA</td>
<td>0.4 [0.3-0.7]</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

DISCUSSION

Patients with NMOSD should receive immunotherapy as early as possible, as any further attack may cause serious and often irreversible disability. However, current knowledge about sequences of immunotherapies, predictors for therapy response or comparison between treatments is limited. The NEMOS database allowed us to analyse ‘real life’ management of NMOSD in a large cohort of 144 patients with NMOSD and an observation period of 321 treatment years.

Patients were cared for at 16 tertiary centres and 5 regional hospitals, therefore covering a study population representative for German patients with NMOSD and the diversity of care. Fifteen different immunotherapeutic drugs were used for the treatment of NMOSD, indicating a heterogeneous treatment of our cohort. The most commonly used drugs were RTX, AZA, IFN, Mitox and GLAT. The high prevalence of drugs regularly used for multiple sclerosis (MS) and now abandoned for NMOSD probably is due to initial misdiagnosis of patients before AQP4-ab testing has become widely available and the fact that inefficacy of these drugs for NMOSD was unknown at that time. As the data closure of this study was already in 2013, data on longer use of RTX and on some of the recently emerging therapies such as TCZ are limited.

Overall, we found moderate to low unadjusted ARRs under RTX and AZA, the two most commonly used drugs for NMOSD in our cohort. This is in line with other studies reporting ARRs under these therapies of between 0.1 and 0.9.

The proportion of patients treated with drugs regularly used for MS therapy declined over the observation period and RTX gained share, in line with its introduction as first-line recommendation for NMOSD in Germany. Of note, while initiation of a drug is guided by recommendations, staying on a drug is influenced by the absence of disease activity and adverse effects. The relative gain of RTX over AZA since 2011 might also be related to its better tolerability.

Using a multivariable model, we compared the effect on disease activity of different immunotherapies. RTX and AZA were the only two drugs that had significantly lower attack rates than IFN. GLAT and Mitox were not superior to IFN. INF with its known lacking efficacy was used as reference as the percentage of untreated patients was too small. The design of our study was not suitable for a direct comparison between AZA and RTX. The appearance of potential similar efficacy should therefore be interpreted with caution and currently cannot be translated into treatment recommendations.
Two previous studies had indeed suggested superiority of RTX over AZA, \(^{22,23}\) While our study included mainly Caucasian patients, these two other retrospective cohorts included African or predominantly Asian patients. \(^{22,23}\) Genetic differences have been previously suggested to contribute to the RTX therapy response in NMOSD. \(^{17}\) Moreover, previous studies had excluded patients with a history of previous immunosuppressive treatment. By contrast, such patients were included in our analyses, which is in line with the fact that most patients with NMOSD are treated with more than one immunosuppressant over the course of disease. \(^{5,22}\) In our cohort, RTX was used in >60% as second-line therapy or even later. Treatment was changed from AZA to RTX in eight patients. This could have generated a bias in favour of a low ARR in the AZA subgroup, as patients who responded well to the treatment might have remained on therapy more often as reported in another cohort; \(^{24}\) in addition, patients with a high ARR under AZA treatment might have been switched to RTX. Moreover, we cannot completely exclude that patients with more aggressive disease were more commonly treated with RTX than AZA based on the presumption of treatment superiority.

Our study identified predictors for therapy response independent from the chosen compound. First, the presence of AQP4-ab was identified as risk factor for attacks under therapy. Post-hoc analyses suggest that this aspect might be more important in RTX-treated than in AZA-treated patients. This is in line with a previous study that did not detect an association between attack risk and AQP4-ab in AZA-treated and MMF-treated patients, but described a decrease of attack risk with age. \(^{46}\) However, the sample sizes are too small to draw final conclusions from our explorative analysis. Second, we found that a previous attack under the same therapy was associated with a 1.5-fold increased risk for further attacks. Therefore, any attack under a sufficiently dosed therapy should stipulate a discussion about alternative treatment regimes, even if the current knowledge about escalation regimes forbids a strict ‘attack equals treatment change’ algorithm. As treatment response in our cohort was completely independent from the line of therapy, suggestions on particular escalation regimes cannot be inferred. Recently, Kim et al \(^{16}\) reported in a retrospective study of 116 patients with NMOSD that non-responders to first-line therapy with AZA or MMF had less relapses on subsequent therapy with RTX. \(^{36}\) However, still larger and longer observations are needed to provide data on meaningful sequences of therapies. The higher risk of attacks in AQP4-ab-positive patients is in line with previous observations in untreated patients \(^{37}\) and indicates that these patients are facing higher inflammatory and more aggressive disease courses than AQP4-ab-negative patients with an NMOSD phenotype. Third, we found a decreased attack risk with increasing age. This suggests that the shorter time to disability milestones in elderly reported in the literature \(^{46}\) is rather driven by worse attack outcome than by higher attack rates. In accordance with this hypothesis, we found a lower remission rate with increasing age in the NEMOS cohort in our previous study. \(^{46}\)

Using a retrospective data set, our study has several limitations. As patients with NMOSD generally require immunotherapy, we could not compare treated and untreated patients; therefore, we used the probably worst performing compound (IFN) as a reference. Second, pretreatment data could not be reliably assessed, which impeded comparison between attack risks before and during first-line treatment of NMOSD. Moreover, we cannot exclude that assignment to drugs regularly used for MS therapy might have been influenced by milder diseases courses. Third, as detailed drug dosing information was not available in all patients, treatment effects could have been underestimated or overestimated. Due to the restricted sample size, we were not able to provide differentiated efficacy estimates for all treatments or subgroup of patients. Finally, as the severity of relapses was not evaluated, differences in efficacy among the drugs could only be assessed with regard to relapse frequency. In future prospective studies, severity of relapses should be recorded.

Although we tried to compensate for other (in this regard confounding) factors when analysing HRs for each therapeutic compound, only randomised, blinded clinical trials can provide highest level evidence for therapy guidelines. However, this is difficult with NMOSD being a rare disease, and thus retrospective analyses of registry data are the currently best available option. Moreover, given the paucity of patients eligible for such prospective interventional clinical trials, it is of paramount importance to sharpen hypotheses to test in such trials using all retrospective data available. National and international collaborative initiatives are needed to engage towards prospective data collection as currently realised for the NEMOS cohort.

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