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Efficacy of different rituximab therapeutic strategies in patients with neuromyelitis optica spectrum disorders

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ABSTRACT

Objective: To evaluate disease activity according to rituximab (RTX) induction and maintenance regimens in a multicenter real-life dataset of NMOSD patients.

Methods: This is an observational-retrospective multicentre study including patients with NMOSD treated with RTX in 21 Italian and 1 Swiss centers. Demographics, relapse rate and adverse events over the follow-up were summarized taking into account induction strategy (two-1 g infusions at a 15-day interval (IND-A) vs. 375 mg/m2/week infusions for one month (IND-B)) and maintenance therapy (regimen A (M-A) with fixed time-points infusions vs. regimen B (M-B) based on cytofluorimetric driven reinfusion regimens, the least further subdivided according to CD19+ B cells (M-B1) or CD27+ memory B cells (M-B2) monitoring).

Results: 131 subjects were enrolled, 127 patients completed the induction regimen and 119 patients had at least one follow-up visit and were included in the outcome analysis. Median follow-up was 1.7 years (range 0.1–11.6). Annualized relapse rate (ARR) was 1.7 in the year before RTX start and decreased to 0.19 during the follow-up. Both ARR and Time to first relapse (TTFR) analysis showed a trend toward an increased disease activity for IND-B and M-A. No patients with MT-B2 experienced relapses during the follow-up. Number of relapses in the year before RTX initiation and having received a previous treatment were significantly associated with higher ARR and reduced TTFR in the multivariate analysis.

Interpretation: We confirm RTX efficacy in NMOSD patients. Use of specific induction and maintenance protocols is warranted in order to foster RTX efficacy and to reduce costs and side effects.

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1. Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is an autoimmune disease mostly characterized by recurrent episodes of optic neuritis and myelitis, alone or in combination (Weinshenker and Wingerchuk, 2017). NMOSD is characterized, in the vast majority of patients, by the presence of auto-antibodies (ab) against aquaporin 4 (AQ4). A small percentage of patients might test positive for myelin oligodendrocyte glycoprotein (MOG) ab, serological markers with putative pathophysiological role (Ramanathan et al., 2018; Hamid et al., 2017). Before introduction of disease modifying treatments, NMOSD prognosis was almost invariably poor, with clinical attacks leading to permanent neurological disability or even death, in few years after onset (Wingerchuk et al., 1999; Wingerchuk and Weinshenker, 2003). There are no approved therapies for NMOSD: patients are treated with relapse-related therapies (*e.g.,* steroids, intravenous immunoglobulin (IVIG) and plasma-exchange) (Trebst et al., 2014) and, with an "off-label" indication, with long term immunosuppressants (*e.g.:* azathioprine, methotrexate, and mycophenolate mofetil (MMF)) (Trebst et al., 2014). Recently a large body of evidence from large retrospective studies (Kim et al., 2015; Radaelli et al., 2016; Torres et al., 2015; Annovazzi et al., 2016) and meta-analysis (Damato et al., 2016) suggested that drugs targeting B cells (*i.e.:* rituximab, RTX) are effective in preventing relapses in NMOSD. As all the other therapeutic approaches in NMOSD, RTX is used off-label, both as a first line therapy, or as a rescue therapy after an ineffective first-line therapy (Ciron et al., 2018). Although RTX is increasingly used in NMOSD, considerable heterogeneity exists, mainly concerning the number and dosage of infusions and the frequency of therapeutic cycles. Pharmaco-economic comparative studies and long-term side effect analyses are still lacking.

Aim of this study is to report a multicentre experience of RTX treatment in patients with NMOSD, comparing efficacy according to patients' characteristics and different regimen strategies, in order to generate hypotheses to be possibly tested in randomized studies.

2. Methods

2.1. Design and setting

We designed a multicenter, retrospective study based on prospectively collected data, involving 21 Italian NMOSD centers and one Swiss center.

2.2. Case definition, inclusion and exclusion criteria

We enrolled all patients that satisfied NMOSD 2015 diagnostic criteria (Wingerchuk et al., 2015) and that started treatment with RTX. Demographic and clinical data were obtained at baseline (date of RTX start); treatment infusions and clinical course data were collected and updated every 6 months. The study enrolment started in October 2015 and the database was locked on April 2018. Anonymized demographic and clinical data were collected from the databases of every center involved and merged in one unified database by one of the authors (IM), and further processed for data cleaning, and analysis.

All patients were tested for anti-AQ4 ab using commercially available assay. Seronegative patients were also tested for anti-MOG ab (only results that derived from tests that were performed on cell-based assays were included). For patients who started RTX before 2015 the data were collected retrospectively and antibodies sero-status was periodically assessed in seronegative patients.

2.3. Procedure

Patient were followed with periodic neurologic assessment, according to local good clinical practice, to detect and report neurological changes and potential adverse events. Patients were also encouraged to contact the center in case of rapid neurological worsening in order to detect potential relapses that may require immediate therapy or MRI scan to confirm exacerbation diagnosis.

2.4. Treatment protocol

RTX infusion protocols were locally chosen by every single center and were consistent for patients enrolled within the same center.

Patients exposed to a long-term steroid therapy and/or that were concomitantly treated with other immunosuppressive agents were excluded from the study.

RTX infusion regimens were classified according to the induction and maintenance regimens protocols applied in the Italian centers as:

Induction:

- Induction regimen A (IND-A): two 1000 mg infusions 15 days apart.
- Induction regimen B (IND-B): four 375 mg/m^2 infusions every week for 4 weeks.

Maintenance:

- Maintenance regimen A (M-A): fixed time-points (6 months) infusions of 1000 mg.
- Maintenance regimen B (M-B): cytofluorimetric based reinfusion regimens.

M-B cytofluorimetric based reinfusion schemes were sub-classified according to the analysed target cell population, as:

- Maintenance regimen B1 (M-B1): 375 mg/m^2 re-infusions based on CD19+ cells reappearance, defined as CD19+ cells exceeding the 1% of peripheral blood mononuclear cells
- Maintenance regimen B2 (M-B2): 375 mg/m^2 re-infusions based on CD27+ memory B (CD19+) cells re-emergence when this population exceeded 0.05% of peripheral blood mononuclear cells in the first 2 years and 0.1% in the following years (Kim et al., 2013).

Patients that were reinfused according to a flow-cytometric based protocol had their blood samples obtained every 12 weeks throughout the duration of the observation period in order to detect CD19+ B cells or CD27+ memory B cells repopulation.

2.5. Outcome measures

The primary outcomes of the study were the annualized relapse rate (ARR), defined as the total number of relapses divided by the total number of patient-years and the time to first relapse (TTFR) over 2 years: a relapse was defined as a new neurological symptom that occurred without fever or signs of infection and the lasted at least for 24 h. In case of worsening of pre-existing deficits, MRI was performed to confirm relapse diagnosis. For brainstem or area postrema syndromes MRI was always warranted.

In order to count relapses after a complete induction treatment we excluded from the analysis the relapses that occurred in the first 3 months after RTX initiation, since RTX maximum efficacy might need several months to occur (Lindsey et al., 2012; Nakashima et al., 2011). Relapses were therefore counted from month 3 after RTX initiation to 24 months (2 years analysis) or to the last available follow-up. Sensitivity analyses including all the relapses were run.

2.6. Safety

We reported all the adverse events (AE) defined as any untoward medical occurrence during RTX treatment, even without a causal relationship with the treatment and the infusion related reactions (IRR), defined as any AE occurring during RTX in-hospital infusions. An AE was considered "serious" if it resulted in any of the following outcomes:

death, a life-threatening AE, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect.

2.7. Statistical analysis

Poisson and Cox regression analyses were used to assess the influence of baseline patients' characteristics and RTX induction and maintenance regimens on ARR and TTFR respectively. TTFR was analysed over the first 2 years after RTX initiation.

Baseline variables included in the models were age at NMOSD onset, sex, disease duration, presence of any comorbidity and autoimmune comorbidities, EDSS, auto ab sero-status, number of relapses in the previous year, active MRI and presence of spinal cord lesions in the year prior to RTX induction, previous treatments. Only factors significantly associated with the outcome at univariate analysis (with a p value < 0.05) were included in a multivariable model with a stepwise procedure (p for inclusion < 0.05).

SAS 9.3 (Institute Inc., Cary, NC, USA) and R statistics version 3.5.0 were used for the computation.

3. Results

3.1. Patients demographics and clinical characteristics

131 patients were enrolled in the study, 127 patients completed the induction regimen and 119 patients had at least one follow-up visit after the induction period (Fig. 1). Complete demographic and clinical characteristics of the cohort are reported in Table 1.

The auto-ab sero-status was known in 105/127 (82.7%) patients while for 22 (17.3%) patients the sero-status was unknown. Out of 105 patients with a known sero-status, 80 (76.2%) patients tested positive for anti-AQ4 ab, 12 patients (11.4%) tested positive for anti-MOG ab and 13 patients (12.4%) were double negative.

Sixty-five (51.2%) patients were treatment naïve; among those who received a previous treatment, azathioprine was the most commonly used drug (53%), followed by MS specific drugs such as fingolimod, natalizumab, glatiramer-acetate or interferons (21%) and other therapies (*i.e.:* MMF, cyclophosphamide and mitoxantrone) (26%). Among those who changed therapy ($N = 62$, 48.8%), reasons for RTX initiation were previous drug inefficacy (55%), lack of tolerance or adverse events (23%) or other reasons (22%). RTX treatment was discontinued in 4 patients during the induction phase (1 infusion related reaction (IRR), 1 death due to neurologic deterioration, 2 due to patient/physician decision).

3.2. Treatment regimens

Eighty patients (63%) followed the IND-A and 43 patients (34%) the IND-B regimen. Four patients (3.1%) infused using other "non-standardized" protocols were not included in the analysis.

The great majority of the patients in our sample received a maintenance regimen M-B ($N = 103$, 87%) while only 16 (13%) patients followed the M-A regimen. Of the 103 patients following the M-B regimen, 16 (15.5%) were infused according to the M-B2 regimen and the remaining 87 (84.5%) according to the M-B1 regimen.

3.3. Relapse activity

Median follow-up, assessed on 119 patients (Fig. 2), was 1.7 years (range 0.1–11.6 years) and the mean interval between infusions, excluding the induction phase, was 8.4 months (standard deviation (SD) ± 4.5). Four patients discontinued RTX during follow-up (switch to azathioprine, MMF, tocilizumab or IVIG) and 3 patients deceased for complications of NMOSD (mean time to death was 3.2 years). The ARR was 0.98 (95% confidence interval (CI):0.86–1.11) in the 2 years and 1.69 (95%CI:1.47–1.93) in the year before RTX initiation; the ARR significantly decreased during RTX treatment to 0.29 (95%CI:0.20–0.41) and 0.28 (95%CI: 0.21–0.37) during year 1 and year 2, respectively ($p < 0.0001$). The ARR during the whole follow up was 0.19 (95%CI:0.15–0.24).

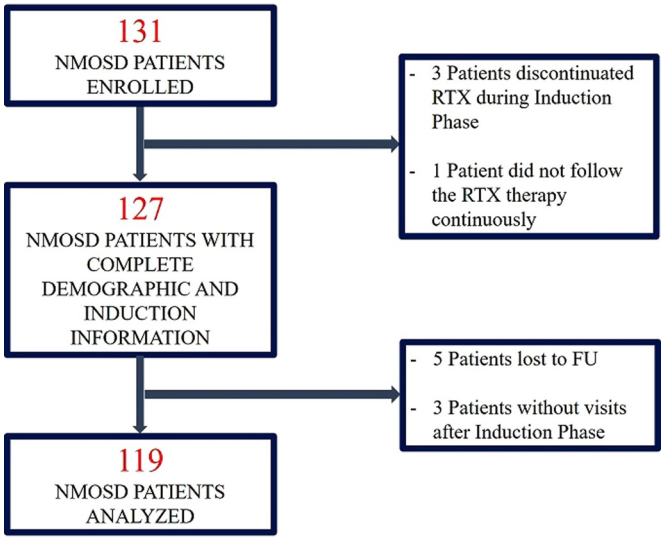

Fig. 1. Flow chart of NMOSD patients enrolled in the study and analysed.

Table 1
Demographical and clinical characteristic of 127 NMOSD patients at Rituximab start.

	All patients $N = 127$
Gender (Female), no. (%)	104 (81.9%)
Auto-ab serostatus, no. (%)	
Anti-AQ4 positive	80/105 (76.2%)
Anti-MOG positive	12/105 (11.4%)
Double negative	13/105 (12.4%)
Age at NMOSD onset, years, mean (± SD)	40.7 (± 14.4)
Age at RTX start, years, mean (± SD)	46.5 (± 14.2)
Disease duration, years, mean (± SD)	5.9 (± 7.2)
Comorbidities, no. (%)	52/108 (48.2%)
Autoimmune comorbidities, no. (%)	34/123 (27.6%)
Number of relapse 2 years before RTX, mean (± SD)	2.1 (± 1.4)
Number of relapse 1 year before RTX, mean (± SD)	1.7 (± 1.0)
EDSS 6 months pre RTX, median (1st-3rd quartiles)	3.5 (2–6)
EDSS at RTX start, median (1st-3rd quartiles)	4 (3–6.5)
Previous treatments, median (range)	0 (0–5)
Naïve patients, no. (%)	65 (51.2%)
Last DMT before RTX, no. (%)	$N = 62$
Azathioprine	33 (53.2%)
MS specific therapy (fingolimod, natalizumab, GA, interferons)	13 (21.0%)
Other	16 (25.8%)
Reason for changing to RTX, no. (%)	$N = 62$
Inefficacy	34 (54.8%)
Intolerance/Adverse events	14 (22.6%)
Other/unknown	14 (22.6%)
Follow-up time since RTX start, years, median (range)	1.7 (0.1–11.6)
	$N = 119$
No. of RTX infusions excluding the first treatment course, median (range)	2 (0–24)
RTX dose/infusions excluding the first treatment course, median (1st-3rd quartile)	1000 (750–1000)
Infusion interval, excluding the first treatment course, months, mean (± SD)	8.4 (± 4.5)

NMOSD: neuromyelitis optica spectrum disorder; AQ4: Aquaporin-4; MOG: myelin oligodendrocyte glycoprotein; SD: standard deviation; RTX: rituximab; EDSS: Expanded Disability Status Scale; DMT: disease-modifying treatment; GA: glatiramer acetate.

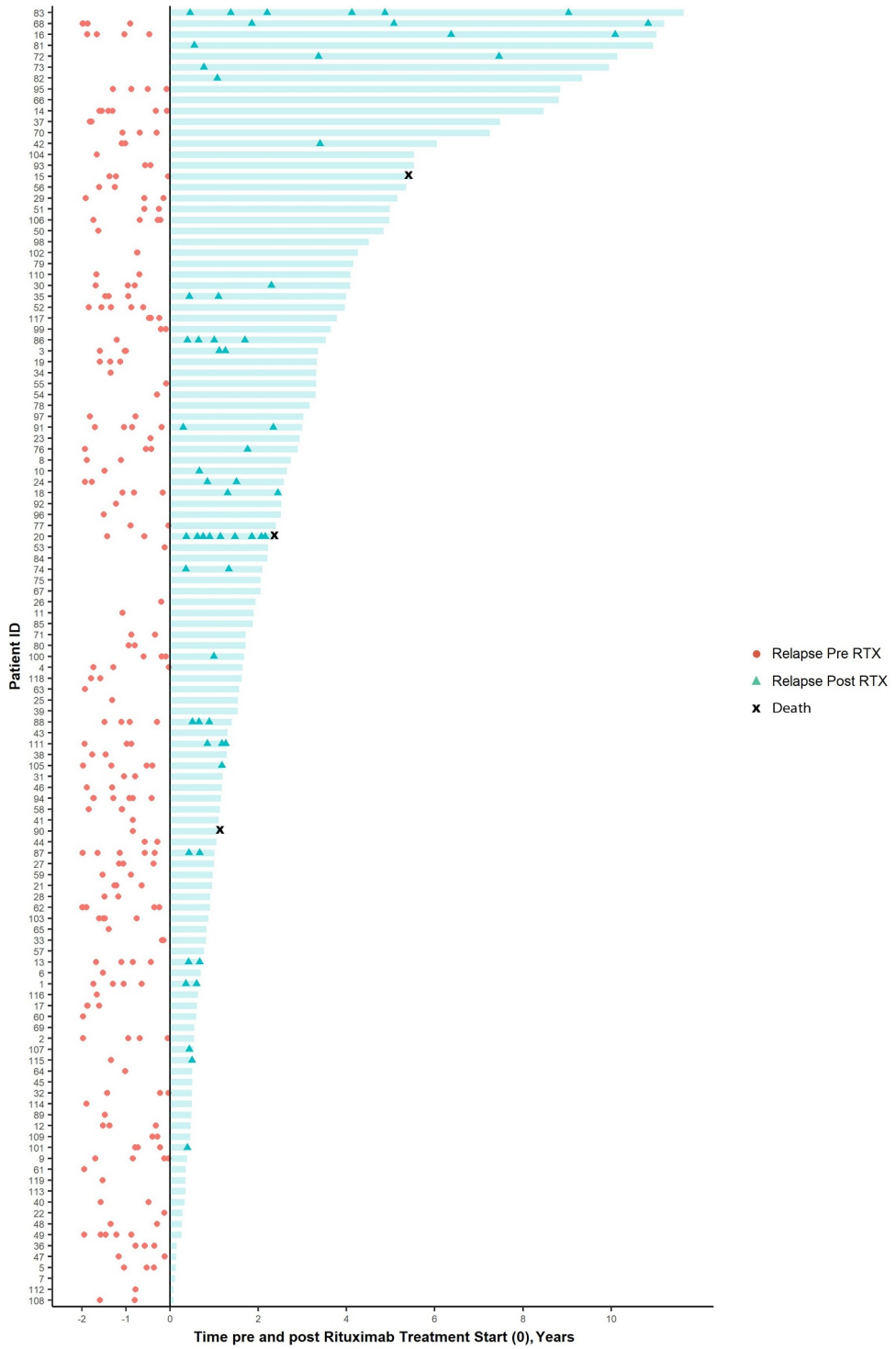

Fig. 2. Relapses in 119 NMOSD patients before and after RTX treatment. The horizontal bars indicate the length of the follow-up; On the horizontal axis, the vertical black bar indicates the start date of RTX treatment and the vertical blue bar indicates the three months after the start date of RTX treatment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

In a sensitivity analysis we also evaluated the impact of the 11 relapses occurring in the first 3 months after RTX initiation: the ARR was 0.40 (95%CI:0.29–0.54) during the first year of RTX maintenance and it was 0.35 (95%CI:0.27–0.45) during the second year ($p < 0.001$). The ARR during the whole follow up was 0.23 (95%CI:0.18–0.28).

A sensitivity analysis excluding 11 anti-MOG positive patients was also performed and no differences in ARR were found. The ARR was 0.36 (95%CI:0.25–0.49) during the first year of RTX maintenance and it was 0.32 (95%CI:0.24–0.42) during the second year. The ARR during the whole follow up was 0.22 (95%CI:0.17–0.27).

Factors associated to the ARR during the RTX treatment at univariate analysis are reported in Table 2.

A higher number of relapses in the year preceding the first RTX induction (rate ratio (RR) = 1.7 (95%CI:1.5–2.0), $p < 0.001$) and being previously treated (RR = 2.5 (95%CI:1.4–5.0), $p = 0.003$) were the factors associated to a higher ARR under RTX (Fig. 3, panel A-B).

Table 2
Comparison of factors associated with 2 years annualized relapse rate and time to first relapse.

	2-Year ARR		2-Year TTFR	
	RR (95% CI)	p-value	HR (95% CI)	*p*-value
Age at NMOSD onset, years	1.0 (1.0–1.0)	0.28	1.0 (1.0–1.0)	0.59
Disease duration, years	1.0 (1.0–1.0)	0.51	1.0 (0.9–1.0)	0.53
Gender (female vs. male)	1.0 (0.2–1.8)	0.97	0.9 (0.3–2.7)	0.91
Presence of comorbidity	0.5 (0.3–1.1)	0.07	0.7 (0.3–1.7)	0.39
Presence of autoimmune comorbidity	1.1 (0.5–2.1)	0.84	0.7 (0.3–2.0)	0.54
EDSS 6 months prior RTX	0.9 (0.8–1.1)	0.46	0.9 (0.7–1.1)	0.20
Number of relapse 1 year before RTX	**1.7 (1.5–2.0)**	**< 0.0001***	**2.0 (1.4–2.7)**	**< 0.0001***
Presence of active MRI 1 year before RTX	1.7 (0.8–3.5)	0.15	1.4 (0.6–3.5)	0.46
Presence of spinal lesion 1 year before RTX	0.8 (0.4–1.6)	0.59	0.6 (0.2–1.4)	0.23
Auto ab serostatus (AQ4 positive as reference)				
Double negative	0.7 (0.3–1.6)	0.36	0.7 (0.2–1.8)	0.17
Anti-MOG positive	1.8 (0.8–4.2)		2.3 (0.7–6.8)	
Treatment history (previously treated vs. naive)	**2.5 (1.4–5.0)**	**0.003***	**2.5 (1.0–5.0)**	**0.05***
Induction regimen ($4 \times 375\,mg/m^2$ vs. $2 \times 1000\,mg$)	1.7 (1.0–3.3)	0.06	1.7 (0.8–5.0)	0.15
Maintenance regimen (fixed vs. cytofluorimetric based)	1.8 (1.0–2.5)	0.12	2.2 (0.8–5.9)	0.10

ARR: annualized relapse rate; TTFR: Time to first relapse; RR: rate ratio; HR: hazard ratio; CI: confidence interval; NMOSD: neuromyelitis optica spectrum disorder; EDSS: Expanded Disability Status Scale; AQ4: Aquaporin-4; MOG: myelin oligodendrocyte glycoprotein.
Factors highlighted in bold were included in the multivariable analysis (those with p value < 0.05 at univariate analysis; Factors marked with an asterisk* are those significant at multivariable analysis retained in the final model; for the multivariable RR and HR see text.

Regarding the treatment regimen impact on ARR, the IND-B induction regimen (RR = 1.7 95%CI:1.0–3.3, $p = 0.06$) and the M-A maintenance regimen (RR = 1.8, 95%CI:1.0–2.5, $p = 0.12$) showed a trend for a higher ARR (Fig. 3, panel C-D).

>Both the numbers of relapses in the previous year (RR = 1.6 (95%CI:1.1–2.3), $p = 0.003$) and having received a previous treatment (RR = 3.3 (95%CI:1.4–5.0, $p = 0.005$) were retained in the multivariable model.

Median TTFR was 1.1 (range 0.1–8.8) years. The univariate analysis (Table 2) showed that the same factors associated to a higher ARR were significantly associated to the TTFR over a 2 years period: the numbers of relapses in the year before RTX induction (hazard ratio (HR) = 2.0, 95%CI:1.4–2.7, $p < 0.001$) and being previously treated (HR = 2.5, 95%CI:1.0–5.0, $p = 0.05$) (Fig. 3, panel E-F). The multivariable analysis retained both factors in the final model: relapse in the previous year (HR = 2.2 (95%CI = 1.5–3.0), $p < 0.0001$) and being previously treated (HR = 2.5 (95%CI = 1.1–5.0), $p = 0.03$).

3.4. Induction and maintenance regimens

As for the treatment regimens there was a trend for a higher relapse risk for the $4 \times 375\,mg/m^2$ induction regimen (IND-B) (HR = 1.7, 95%CI:0.8–5.0, $p = 0.15$) and for the maintenance regimen at fixed timepoints (M-A) (HR = 2.2, 95%CI:0.8–5.9, $p = 0.10$) (Fig. 3, panel G-H).

Additionally, analysing the three protocols based on fixed timepoints reinfusions (M-A), cytofluorimetric reinfusion (CD19+ cells (M-B1) and on CD27+ memory B cells reappearance (M-B2)), described in Methods section, we realized that none of the patients infused according to M-B2 experienced relapses during the whole FU (Fig. 4) and, of note, patients were reinfused at a lower frequency (*i.e.:* with longer infusion intervals) and with a reduced infusion dosage (Table 3).

Due to the small sample size of the subgroups and the fact that the M-B2 regimen was used in only 1 center (Genoa), the p-value ($p = 0.04$) for the comparison (Fig. 4) must be taken with caution.

3.5. Adverse events

During the whole follow-up, 35 patients (29.4%) experienced at least one IRR (mainly skin rash), while 4/35 patients (11.4%) experienced a serious IRR (skin rash, difficulty breathing, syncope and neutropenia). Twenty-four patients (20.2%) experienced at least one AE and 8/24 patients (33.3%) experienced at least one serious AE. Urinary tract infections and respiratory infections were the most common AE reported in our sample.

Three patients (2.5%) deceased for complications of NMOSD. Two of them followed the IND-B regimen and were maintained according to the M-B regimen and one of them followed the IND-A and the M-A regimen; none of them were treatment naïve. No cases of opportunistic infections nor cases of progressive multifocal leukoencephalopathy were reported during the follow-up.

4. Discussion

In this large observational study run in many different centers we confirm RTX efficacy in preventing attacks in patients with NMOSD. We enrolled patients from 21 Italian and 1 Swiss centers that have been treated with RTX and that completed at least the induction regimen. Demographic and clinical characteristics of enrolled patients are in line with previous literature reports, showing a high prevalence of woman among NMOSD patients, often with autoimmune co-morbidities (mainly autoimmune thyroiditis, type 1 diabetes and undifferentiated connective-tissue disease) and with higher age of disease onset, compared to the prototypical MS population (Wingerchuk et al., 2007). We also showed that a high proportion of patients present with anti-AQ4 ab and that a minority of patients (comparable to published papers (Hamid et al., 2017)) tested positive for anti-MOG ab. In our study the frequency of attacks and the time to first attack under RTX therapy appeared to be affected by the treatment regimens: the induction regimen showed a better performance of the two 1000 mg infusions 15 days apart vs. the four $375\,mg/m^2$ infusions every week for 4 weeks.

Such results are in line with a previous Italian-based study (Annovazzi et al., 2016), in which IND-A showed to be more effective in reducing disease activity, with results that reached statistical significance. In our study we observed only a trend that favours IND-A, even though the sample size was bigger. Possibly, such discrepancy is due to a higher disease activity in the patients enrolled in the 2016 study (*i.e.:* ARR in the year before RTX 2.3 ± 1.3 vs. 1.7 + 1.0 of the present study).

The maintenance regimen analysis showed a better performance of the cytofluorimetric based reinfusion regimens vs. the fixed time points reinfusion regimens, even if the associations did not reach the statistical significance.

In particular, the re-infusion protocol based on CD27+ memory B cells assessments had a very good performance, with no observed

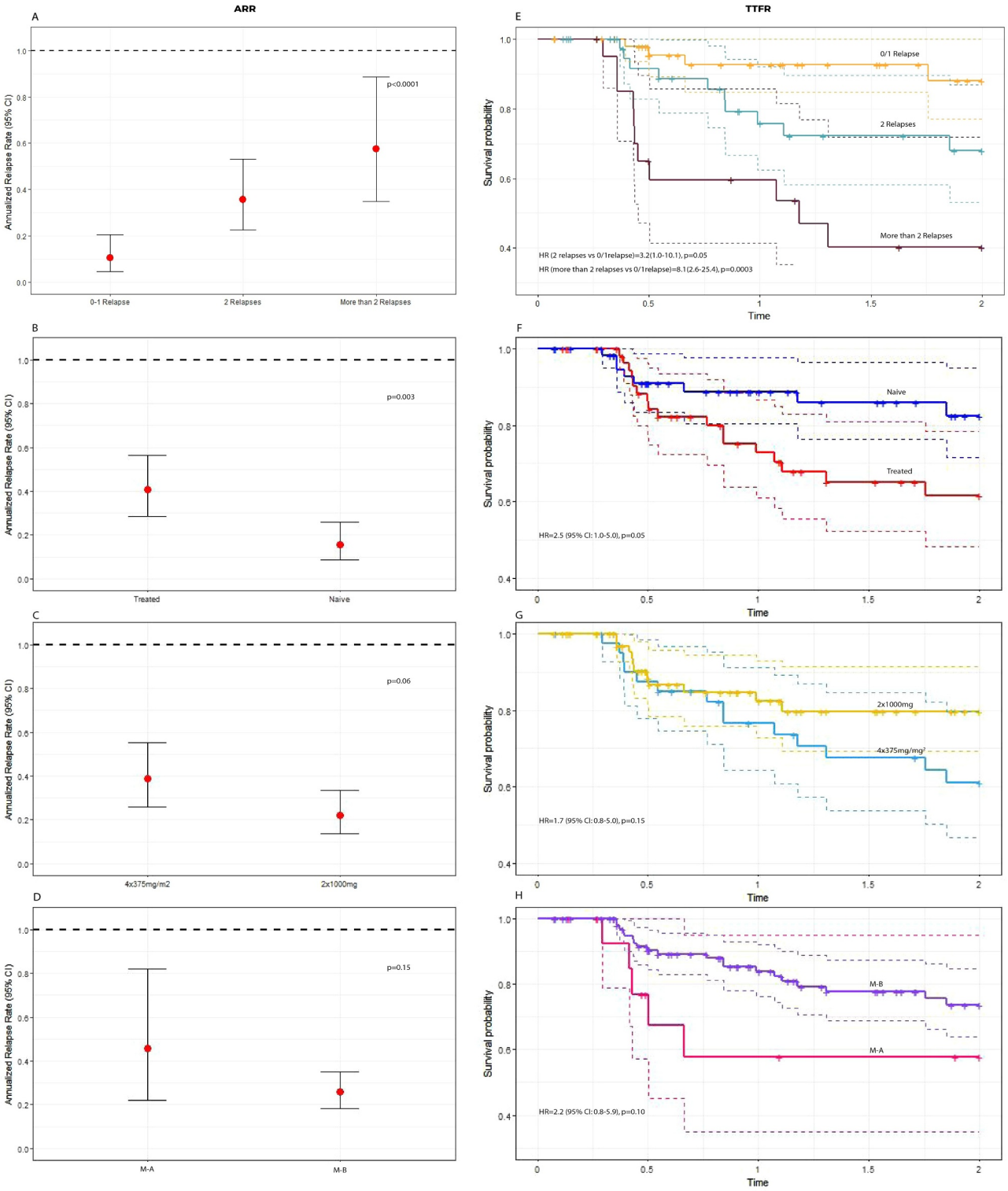

Fig. 3. 2-year ARR and TTFR according to the number of relapses in the year preceding the first RTX induction (panels A and E), to the patient treatment history (panels B and F), to the induction regimes (panels C and G) and to the maintenance regimens (panels D and H). ARR: annualized relapse rate; TTFR: time to first relapse; HR: hazard ratio; CI: confidence intervals. In panels from A to D *p*-values were obtained from a Poisson regression model with the number of relapses in the year preceding the first RTX induction as only factor (panel A), the patient treatment history as only factor (panel B), the induction regimes as only factor (panel C) and to maintenance regimens as only factor (panel D). In Panels from E to G, Kaplan–Meier curves were represented and HRs and *p*-values of the Log-rank test were reported.

relapses in the subgroup who underwent this regimen. Additionally, as previously shown (Cohen et al., 2017), patients treated according to this protocol had a lower frequency of infusions as compared to the other groups, with longer infusion intervals and lower infusion dosage (and possibly reduced costs). Short clinical follow-up might represent the study main limit. However, if our observation will be confirmed in cohorts with longer follow up duration, it could become relevant in the choice of RTX regimen for the treatment of NMOSD patients.

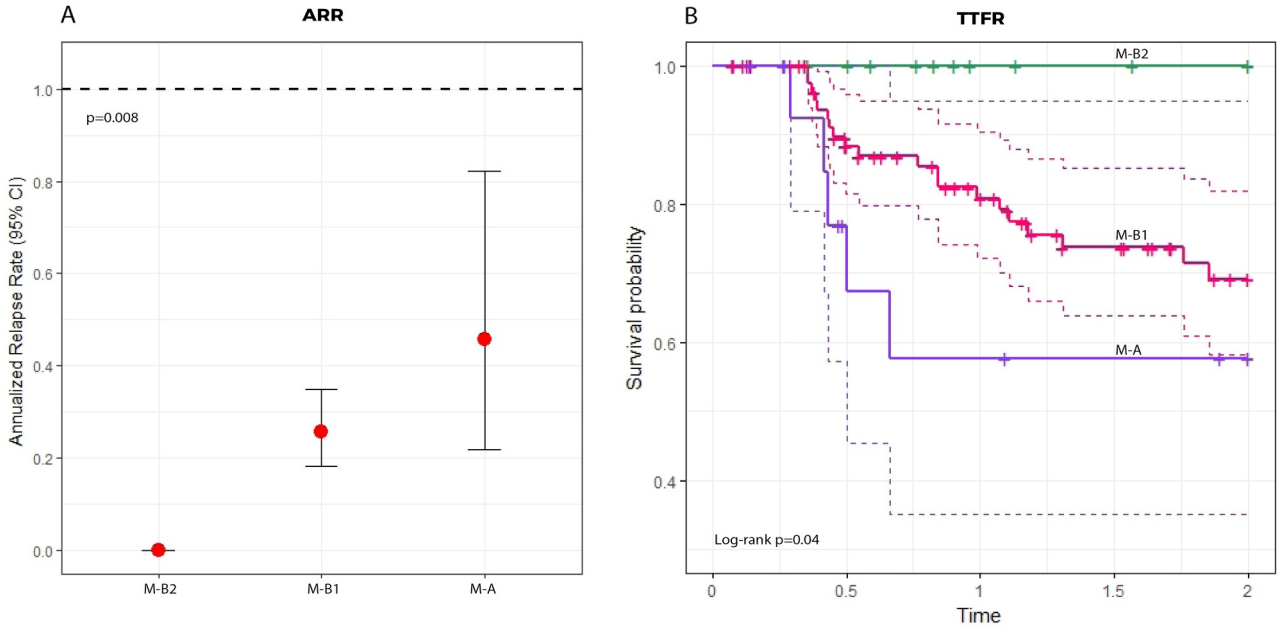

Fig. 4. 2-year ARR (Panel A) and TTFR (Panel B) according to maintenance regimens. M-A: fixed timepoint infusion regimen using 6-months infusions of 1000 mg; M-B1: re-infusions based on CD19+ cells reappearance; MR-B2: re-infusions based on CD27+ cells reappearance. ARR: annualized relapse rate; TTFR: time to first relapse; CI: confidence intervals; In panel A *p*-value was obtained from a Poisson regression model with the maintenance regimen as the only factor. In Panel B Kaplan–Meier curves of the three maintenance regimens were represented and *p*-value of the Log-rank test of a general association was reported.

Table 3
Rituximab maintenance according to maintenance regimens.

	MR-A $N = 16$	MR-B1 $N = 87$	MR-B2 $N = 16$
No. of RTX infusions, median (range)*	2 (0–12)	2 (0–24)	1 (0–6)
RTX dose/infusion, mg, median (1st-3rd quartile)*	1000 (1000–1000)	1000 (738.3–1000)	750 (750–750)
Infusion interval, days, mean (SD)*	188 (67)	261 (151)	294 (74)
Follow-up since RTX start, years, median (range)	1.2 (0.1–8.8)	1.7 (0.1–11.6)	1.4 (0.4–5.3)

MR-A: a fixed timepoint infusion regimen using 6-months infusions of 1000 mg; MR-B1: re-infusions based on CD19+ cells reappearance; MR-B2: re-infusions based on CD27+ cells reappearance.
* Excluding the first treatment course.

The higher relapse rate observed in the MR-A group is unexpected since, in such group infusions were more frequent and with higher dosage. Probably, kinetic in B cells repopulation (*e.g.,* expression of CD20+) and in RTX elimination could explain the different efficacy.

Use of tailored therapies schemes is warranted in order to optimize treatment efficacy, reduce possible side-effects and limit treatment-related costs. In this setting, a tailored maintenance regimen based on CD27 memory B cells reappearance seems to be able to optimize RTX efficacy, without increasing treatment related side effects and possibly reducing treatment-related costs (due to higher infusion interval and lower dosage). Additionally, use of IND-A instead in IND-B can be advised in order to reduce cumulative dose of RTX and further reduce treatment related costs.

We also showed that patients that were treatment naïve performed better than patient that were already treated. This result could be explained by two factors: firstly, it has been already reported that some MS approved therapy (*e.g.,* fingolimod or natalizumab) might induce a paradoxical burst in disease activity in NMOSD (Min et al., 2012; Jacob et al., 2012) but it's not known whether the effects abates after therapy discontinuation. Secondly, a selection bias might apply since "switching" patients might have higher baseline activity than naïve patients. Finally, longstanding (*i.e.:* "switching") NMOSD patients might experience a higher disease activity due to modification in disease pathophysiology (*e.g.,* epitope spreading or production of long-living plasmablasts that do not express CD20 molecule).

RTX efficacy in presence of anti-MOG ab has not yet been compared to the typical (anti-AQ4 positive or double seronegative) NMOSD population. In our sample we found a trend toward an increased efficacy of RTX in double seronegative patients and a reduced efficacy in anti-MOG ab positive patients. However, both in the univariate and multivariable analysis the associations were not statistically significant, also due to the limited number of patients in these two subgroups.

Lastly, it is of outmost importance that no treatment associated adverse events occurred or were related to different induction or maintenance regimen. Additionally, no cases of opportunistic infections, especially progressive multifocal leukoencephalopathy, were reported, confirming safety of CD20 depleting therapies in NMOSD population.

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