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ARTICLE

# Autologous nonmyeloablative hematopoietic stem cell transplantation for neuromyelitis optica

Richard K. Burt, MD, Roumen Balabanov, MD, Xiaoqiang Han, MD, Carol Burns, RN, Joseph Gastala, MD, Borko Jovanovic, PhD, Irene Helenowski, PhD, Jiraporn Jitprapaikulsan, MD, James P. Fryer, MS, and Sean J. Pittock, MD

**Correspondence**
Dr. Burt
rburt@northwestern.edu

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## Abstract

### Objective

To determine if autologous nonmyeloablative hematopoietic stem cell transplantation (HSCT) could be a salvage therapy for neuromyelitis optica spectrum disorder (NMOSD).

### Methods

Thirteen patients were enrolled in a prospective open-label cohort study (11 NMOSD aquaporin-4–immunoglobulin G [AQP4-IgG]–positive, 1 NMOSD without AQP4, and 1 NMOSD AQP4-IgG-positive with neuropsychiatric systemic lupus erythematosus [SLE]). Following stem cell mobilization with cyclophosphamide (2 g/m$^2$) and filgrastim, patients were treated with cyclophosphamide (200 mg/kg) divided as 50 mg/kg IV on day −5 to day −2, rATG (thymoglobulin) given IV at 0.5 mg/kg on day −5, 1 mg/kg on day −4, and 1.5 mg/kg on days −3, −2, and −1 (total dose 6 mg/kg), and rituximab 500 mg IV on days −6 and +1. Unselected peripheral blood stem cells were infused on day 0. AQP4-IgG antibody status was determined by Clinical Laboratory Improvement Amendments–validated ELISA or flow cytometry assays. Cell-killing activity was measured using a flow cytometry–based complement assay.

### Results

Median follow-up was 57 months. The patient with coexistent SLE died of complications of active lupus 10 months after HSCT. For the 12 patients with NMOSD without other active coexisting autoimmune diseases, 11 patients are more than 5 years post-transplant, and 80% are relapse-free off all immunosuppression ($p < 0.001$). At 1 and 5 years after HSCT, Expanded Disability Status Scale score improved from a baseline mean of 4.4 to 3.3 ($p < 0.01$) at 5 years. The Neurologic Rating Scale score improved after HSCT from a baseline mean of 69.5 to 85.7 at 5 years ($p < 0.01$). The Short Form–36 health survey for quality of life total score improved from mean 34.2 to 62.1 ($p = 0.001$) at 5 years. In the 11 patients whose baseline AQP4-IgG serostatus was positive, 9 patients became seronegative by the immunofluorescence or cell-binding assays available at the time; complement activating and cell-killing ability of patient serum was switched off in 6 of 7 patients with before and after HSCT testing. Two patients remained AQP4-IgG-seropositive (with persistent complement activating and cell-killing ability) and relapsed within 2 years of HSCT. No patient with seronegative conversion relapsed.

### Conclusion

Prolonged drug-free remission with AQP4-IgG seroconversion to negative following nonmyeloablative autologous HSCT warrants further investigation.

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From the Division of Immunotherapy, Department of Medicine (R.K.B., X.H., C.B.), and Departments of Neurology (R.B.), Radiology (J.G.), and Preventive Medicine (B.J., I.H.), Northwestern University Feinberg School of Medicine, Chicago, IL; and the Departments of Neurology (J.J., S.J.P.) and Laboratory Medicine and Pathology (J.J., J.P.F., S.J.P.) and Center for Multiple Sclerosis and Autoimmune Neurology (S.J.P.), Mayo Clinic College of Medicine, Rochester, MN.

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## Glossary

**AQP4** = aquaporin-4; **CI** = confidence interval; **CMV** = cytomegalovirus; **EBMT** = European Bone Marrow Transplant; **EDSS** = Expanded Disability Status Scale; **HSCT** = hematopoietic stem cell transplantation; **IgG** = immunoglobulin G; **LETM** = longitudinally extensive transverse myelitis; **MOG** = myelin oligodendrocyte glycoprotein; **MS** = multiple sclerosis; **NMO** = neuromyelitis optica; **NMOSD** = neuromyelitis optica spectrum disorder; **NRS** = Neurologic Rating Scale; **PFS** = progression-free survival; **PI** = propidium iodide; **rATG** = rabbit antithymocyte globulin; **RFS** = relapse-free survival; **SF-36** = Short Form–36; **SLE** = systemic lupus erythematous; **URTI** = upper respiratory tract infections.

Neuromyelitis optica spectrum disorder (NMOSD) is recognized as a distinct demyelinating disease with a worse prognosis, unique biomarker, and different imaging features, pathogenesis, and response to therapy than multiple sclerosis (MS).[1–7] Longitudinally extensive transverse myelitis (LETM) involves the central (ependymal) canal of the spinal cord and is continuous over 3 or more vertebral sequences.[5,6] Optic neuritis often involves the posterior optic nerves or chiasm.[5–7] Brain lesions are typically periependymal or brainstem (medulla, pons, diencephalon) in location with typically few cerebral lesions until late in disease evolution.[5,6]

Five years after diagnosis of NMOSD, 50% of patients are legally blind in one or both eyes or walking impaired.[4] NMOSD is associated with an antibody directed against aquaporin-4 (AQP4), a water channel protein that is found on astrocytes.[4] AQP4–immunoglobulin G (IgG) antibodies activate complement, cause cell death in ex vivo assays, are not present in MS, and antibody titers have been reported to correlate with disease activity.[8–10] Over time, the sensitivity and specificity of AQP4-IgG assays improved to better identify AQP4-IgG-positive individuals.[11]

NMOSD includes AQP4-IgG-seronegative subtypes in which approximately 50% are myelin oligodendrocyte glycoprotein (MOG)–IgG-seropositive,[12] and patients with systemic lupus erythematous (SLE) who are both antinuclear antibody– and aquaporin-4-seropositive.[13] The MRI of patients with SLE with anti-AQP4 antibody may demonstrate a distinct imaging pattern with extensive cerebral white matter lesions, and the AQP4-IgG serostatus in patients with SLE has not been reported to correlate with disease activity.[13,14] While part of NMOSD criteria, anti-MOG disease and neuropsychiatric lupus with anti-AQP4 antibody may be distinct diseases. In this pilot study, we tried to minimize potential heterogeneity in diagnosis by focusing on anti-AQP4-seropositive patients with classic MRI.

Pharmaceutical drugs approved for MS are generally ineffective and, in some cases, may aggravate disease symptoms.[15–17] Current immunosuppressive drugs,[18–22] IV immunoglobulin,[23] plasmapheresis,[24] or complement inhibition with eculizumab[25] do not cure, and patients remain on maintenance immunosuppression indefinitely between acute neurologic attacks. We undertook this study to determine if autologous hematopoietic stem cell transplantation could be an effective one-time treatment for NMOSD.

## Methods

### Standard protocol approval, registration, and consent

The trial was approved by the institutional review board (ClinicalTrials.gov identifier: NCT00787722) and enrolled from 2008 through 2016. All patients signed informed consent.

### Patients

One patient was removed from the study due to severe active SLE with pulmonary and renal involvement and atypical MRI with no spinal involvement and no posterior optic or chiasm lesions (only anterior optic neuritis) with large diffuse demyelinating cerebral lesions. Patients were 18–65 years old and were required to fulfill diagnostic criteria for NMOSD with a relapsing course (more than one attack), LETM or optic neuritis (preferably both), and after enrolling the first 4 patients, seropositivity for neuromyelitis optica (NMO)–IgG AQP4-IgG by a Clinical Laboratory Improvement Amendments–approved assay. Patients had to be able to demonstrate ability to ambulate with at least a walker (Expanded Disability Status Scale [EDSS] ≤ 7.0)[26] and were excluded for paraplegia or quadriplegia.

### Stem cell collection and transplant regimen

Peripheral blood stem cells were collected 10 days after IV cyclophosphamide (2 g/m$^2$) and subcutaneous filgrastim (5–10 μg/kg/d) beginning 5 days after cyclophosphamide. The day before hospital admission for transplantation, patients underwent outpatient plasmapheresis. The immune ablative regimen was IV cyclophosphamide 50 mg/kg/daily on day −5 to day −2 before stem cell infusion (day 0), rabbit antithymocyte globulin (rATG) 0.5 mg/kg on day −5, 1.0 mg/kg on day −4, and 1.5 mg/kg on days −3, −2, and −1, and rituximab (500 mg) on days −6 and +1. IV methylprednisolone (1,000 mg) was infused 30 minutes prior to rATG infusion.

### Supportive care guidelines

Blood products were irradiated, cytomegalovirus (CMV)–safe, and leukocyte-depleted. Filgrastim (5–10 μg/kg/d) was started on day +4 and continued until engraftment. Hydration (125–150 mL normal saline per hour), diuretics, and IV mesna were continued until 24 hours after the last dose of cyclophosphamide. An indwelling urinary Foley catheter was placed in patients with greater than 60 mL postvoid urinary

residual. IV cephalosporin was started on day 0. IV vancomycin was added for a febrile episode. Oral acyclovir was started on admission and continued for 1 year. Oral fluconazole was started on day +2, and oral trimethoprim–sulfamethoxazole or monthly nebulized pentamidine was started after platelet engraftment and continued for 6 months. CMV viral load was monitored for 90 days and was treated preemptively by switching from acyclovir to oral valganciclovir (900 mg twice daily) until negative by quantitative PCR.

### Study endpoints

Two primary endpoints were evaluated: relapse-free and progression-free survival (PFS) defined as worsening of the EDSS score[26] by an increase of ≥1 point sustained for 6 months. Secondary endpoints included EDSS (range 0–10 in 0.5 increments from none [0] to worst [10] neurologic disability), Neurologic Rating Scale (NRS)[27] (range 0–100 in 1-point increments from worst [0] to no [100] disability), and quality of life Short Form–36 (SF-36). Relapses were defined as neurologic symptoms lasting more than 24 hours, not associated with infection, fever, or heat intolerance, and deemed to require treatment. Patients returned at 6 months, 1 year, and yearly for 5 years. If a patient could not return for scheduled visit, a telephone conference was scheduled.

### Detection of AQP4-IgG and titer measurement

Stored serum samples were tested at Mayo Clinic (Rochester, MN), which developed the M1-AQP4-transfected cell-based flow cytometry assays.[28] Samples were screened at 1:5 dilution and, if positive, retested at a dilution of 1:5, 1:10, and titrated further in 10-fold dilution steps. The farthest dilution yielding a positive result for IgG binding index ≥ 2.0 was recorded as the endpoint of positivity.

### AQP4-IgG complement study methods

All sera were tested for AQP4-IgG-linked complement activation using a flow cytometry–based assay developed in-house utilizing a stable cell line expressing AQP4-M23. Heat-inactivated patient serum (56°C, 35 minutes) was serially diluted (doubling) from 1:5 out to 1:100,000 in line cell binding buffer. For analysis, 50 μL of each dilution was added to live AQP4-M23 cells. After 10 minutes, 18 μL of rabbit Lo-Tox complement (Cedarlane, Burlington, Canada) was added and the plate was held at room temperature for 30 minutes. Buffer B supplemented with 75 nM EDTA and 0.5 μg propidium iodide (PI; Becton Dickinson, Franklin Lakes, NJ) was added and held for 15 minutes in the dark. An additional 100 μL Buffer B–EDTA without PI was added prior to analysis by flow cytometry. Negative controls were tested on every plate and were the basis for determining positive populations. The percent of positive events was used to evaluate the level of complement activation and cell killing.

### Statistical analysis

Time to progression and time to relapse were estimated via the Kaplan-Meier method and assessed via the log-rank test. Differences in outcome measures (EDSS, NRS, and SF-36) were compared by 2-tailed paired *t* test.

### Data availability

Individual de-identified participant data will not be available in a publicly accessible repository to protect the interests of the patients and investigators.

## Results

### Demographics

Twenty-two patients were referred for hematopoietic stem cell transplantation (HSCT). Nine patients were omitted from the study: 5 for advanced neurologic disability (paraplegia or quadriplegia), 2 for comorbid diseases (1 coronary artery and cerebrovascular disease, 1 narcotic addiction), and 2 for being persistently AQP4-IgG-negative. One patient, who was AQP4-IgG-seropositive with refractory antinuclear antibody–positive SLE complicated by glomerulonephritis, nephrotic syndrome, pulmonary hemorrhage, colitis, and neuropsychiatric lupus with seizures and extensive white matter cerebral lesions without LETM or optic chiasm atrophy, subsequently died 10 months after HSCT from lupus-related alveolar hemorrhage. Thereafter, the study was modified to exclude coexisting SLE.

Of the 12 patients with NMOSD without other active comorbid diseases, 11 were female, 8 were Caucasian, and 4 were African American. The mean age was 42 years (range 19–51). Mean duration of disease before HSCT was 84 months (range 11–236 months), mean number of acute neurologic relapses was 4.3 (range 2–10), and mean number of different immune treatments was 4 (range 2–6). Before transplant, 11 had clinical attacks of optic neuritis, 12 had myelitis, 1 had area postrema syndrome, and 11 of 12 were AQP4-IgG-positive. The mean pretransplant EDSS was 4.3 (range 2–6.5) (table 1).

Pretransplant MRI demonstrated LETM within the thoracic spine in 100% (12/12) of patients and within the cervical spine in 50% (6/12) of patients. In all cases of LETM, demyelination was centrally located around the ependymal (central) canal. Two patients had brainstem (medulla, pons) lesions, 2 had periependymal fourth ventricle lesions, and 1 had extensive atrophy of the corpus callosum. Periventricular, subcortical, and deep white matter cerebral lesions were small (≤5 mm) in all cases except one (1.7 cm), generally infrequent, and inconsistent with a diagnosis of MS or vasculitis. Atrophy of the optic chiasm was present on MRI in 50% (6/12), with 4 demonstrating unilateral and 2 bilateral optic atrophy.

### Toxicity

The median day of white blood cell engraftment (absolute neutrophil count >1,000/μL) and hospital discharge after HSCT was day +9 and +10, respectively. The only inpatient

**Table 1** Patient demographics

| Patient | Ethnicity | Sex | Age, y | AQP4-IgG | Disease duration before HSCT, mo | Follow-up time since HSCT, mo | Acute attacks pre HSCT | Time (months before HSCT) of relapses 2 years before HSCT | Pre HSCT immune-modulating medication | Post HSCT immune-modulating medication and relapse | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 6 mo | 12 mo | 24 mo | 36 mo | 48 mo | 60 mo |
| **1** | White | M | 51 | Positive | 43 | 60 | 4 | −23, −12, −6 | Betaseron, Rebif, steroids, PLEX, Rituxan | N | N | N | N | N | N |
| **2** | Black | F | 41 | Positive | 14 | 60 | 3 | −14, −3 | Interferon, Cytoxan, Imuran, steroids | N | N | N | N | N | N |
| **3** | White | F | 50 | Positive | 11 | 60 | 2 | −9 | PLEX, steroids, Imuran | N | N | N | N | N | N |
| **4** | Black | F | 45 | Positive | 132 | 60 | 2 | N | IV steroids, Avonex, Copaxone, Rituxan | N | N | Relapse/ PLEX, steroids, rituximab | N | PLEX | N |
| **5** | White | F | 38 | Negative | 126 | 60 | 3 | −13 | Steroids, Rituxan | N | N | N | N | N | N |
| **6** | White | F | 34 | Positive | 53 | 60 | 3 | −5 | Steroids, Imuran, PLEX, Rituxan | N | N | N | N | N | N |
| **7** | Black | F | 51 | Positive | 156 | 60 | 9 | −10, −3 | IV steroids, PLEX, Imuran, CellCept | N | N | N | N | N | N |
| **8** | White | F | 34 | Positive | 18 | 60 | 5 | −16, −7, −4, −3 | Steroids, Copaxone | | | Relapse/ steroids | N | N | Cytoxan |
| **9** | White | F | 43 | Positive | 236 | 60 | 3 | N | IVIg, steroids, Rituxan, Tysabri, Gilenya | N | N | N | N | N | N |
| **10** | Black | F | 49 | Positive | 62 | 60 | 4 | −22, −19 | Steroids, CellCept, PLEX, Rituxan | N | N | N | N | N | N |
| **11** | White | F | 46 | Positive | 142 | 54 | 11 | −8 | IVIg, steroids, PLEX, Rebif, Rituxan, Imuran | N | IVIg (infection prophylaxis) | IVIg (infection prophylaxis) | N | N | N |
| **12** | White | F | 19 | Positive | 11 | 24 | 2 | −9 | Steroids, PLEX, Rituxan | Steroids, Neupogen for neutropenia | N | N | | | |
| **Summary, average (range)** | 8 White, 4 black | 1 M, 11 F | 42 YO (19–51) | 1 Negative, 11 positive | 84 mo (11–236) | 56.5 mo (24–60) | 4.3 (2–10) | Total: 18 | | | | | | | |

Abbreviations: AQP4 = aquaporin-4; HSCT = hematopoietic stem cell transplantation; IgG = immunoglobulin G; IVIg = IV immunoglobulin; PLEX = plasmapheresis.
Not included is one patient who was AQP4-seropositive with refractory antinuclear antibody–positive systemic lupus erythematosus complicated by glomerulonephritis, nephrotic syndrome, pulmonary hemorrhage, colitis, and neuropsychiatric lupus with seizures and extensive white matter cerebral lesions without longitudinally extensive transverse myelitis or optic chiasm atrophy.

infection was 1 case of *Clostridium difficile* diarrhea. There were no grade 4 toxicities. Grade 3 toxicities and number of patients afflicted were hypophosphatemia (9), neutropenic fever (5), hypocalcemia (3), nausea and vomiting (3), hypokalemia (2), orthostatic hypotension (2) and 1 each of hypertension, hyperglycemia, and diarrhea. Infectious events and number of patients affected over the 5-year interval after hospital discharge for HSCT were *C difficile* diarrhea (2), urinary tract infection (2), upper respiratory tract infections (URTI) including pneumonia (3), and 1 each of streptococcus pharyngitis, dermatomal zoster, and influenza. One patient was treated transiently with IV immunoglobulin for recurrent URTI and hypogammaglobulinemia. The number of infections after HSCT was 0.18 per year per patient. Other post-transplant events were 1 cholecystectomy, 2 shoulder surgeries, delivery of a healthy baby, and 2 new autoimmune diseases (1 case of myasthenia gravis that occurred with NMOSD relapse and 1 case of hyperthyroidism).

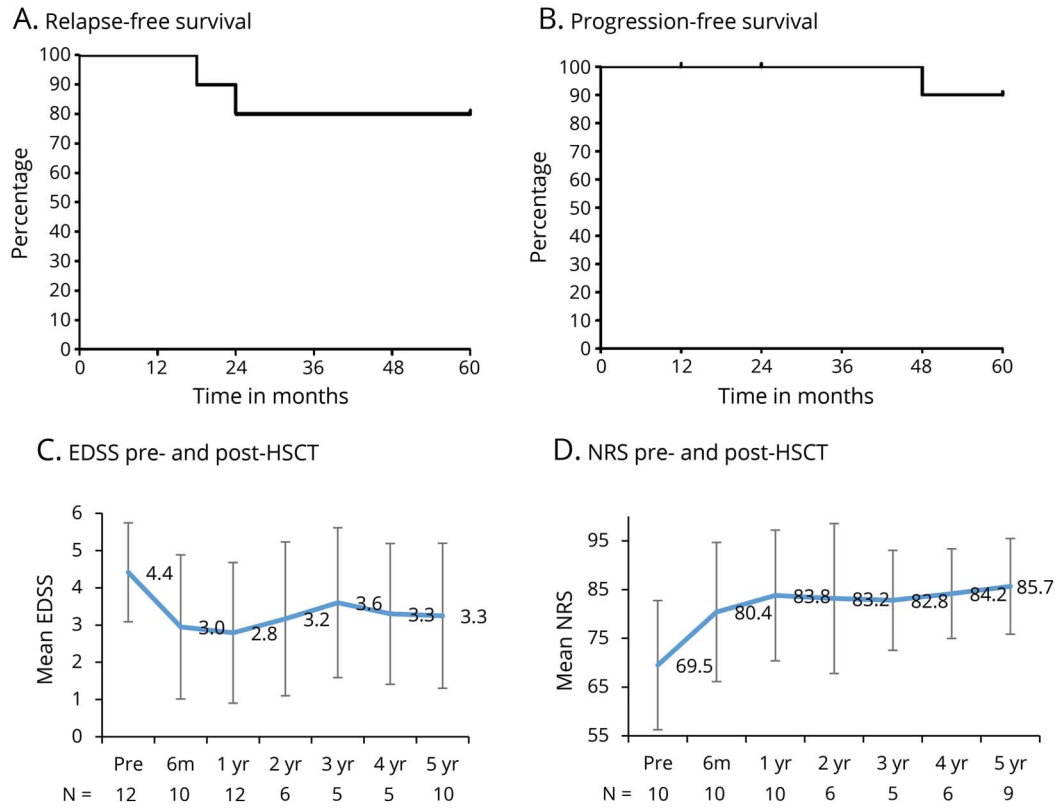
## Outcome

Excluding the patient with refractory SLE, PFS at 5 years was 90% (confidence interval [CI] 47%–98%) (figure 1). Relapse-free survival (RFS) at 5 years was 80% (CI 41%–94%). EDSS improved from a pretransplant mean of 4.4 (median 4.3, range 2–6.5) to 2.8 (median 1, range 1–6.5) ($p = 0.004$) at 1 year and 3.3 (median 3.0, range 0–6.5) at 5 years ($p = 0.03$). The NRS improved from a pretransplant mean of 69.5 (median 68, range 48–89) to 85.7 (median 86.5, range 72–100) at 5 years ($p = 0.001$).

Mental, physical, and total SF-36 quality of life significantly improved following HSCT (table 2). After HSCT, the mental SF-36 mean improved significantly from 37.6 to 59.5 at 1 year and 65 at 5 years. The physical SF-36 mean improved significantly from 28 to 51 at 1 year and 56 at 5 years. The total SF-36 mean improved significantly from 34.2 to 55 at 1 year and 62 at 5 years (table 2). The number of patients who required assistance for ambulation went from 6 of 12 pre-HSCT to 2, 2, 2, 1, 1, and 1 at 6 months, 1, 2, 3, 4, and 5 years post transplantation, respectively (figure 2).

A single patient was AQP4-IgG-seronegative pre-transplantation. In the remaining 11 AQP4-IgG-seropositive pre-HSCT patients, 9 seroconverted to negative by the assays available at the time (table 3) and 7 converted to seronegative by the highly sensitive and specific FACS assay retrospectively performed in some cases on stored serum samples (table 3 and figure 3). All seroconverters regardless of AQP4-IgG assay remained relapse-free at last follow-up. The 2 patients who

**Figure 1** Neurologic outcome after hematopoietic stem cell transplantation (HSCT) for neuromyelitis optica spectrum disorder




(A–D) EDSS = Expanded Disability Status Scale (range 0–10 in 0.5 increments from none [0] to worst [10] neurologic disability). NRS = neurologic rating scale (range 0 [worse]–100 [best] in 1-point increments).

**Table 2** Quality of life (QOL) Short Form–36 (SF-36) scores before and after hematopoietic stem cell transplantation (HSCT)

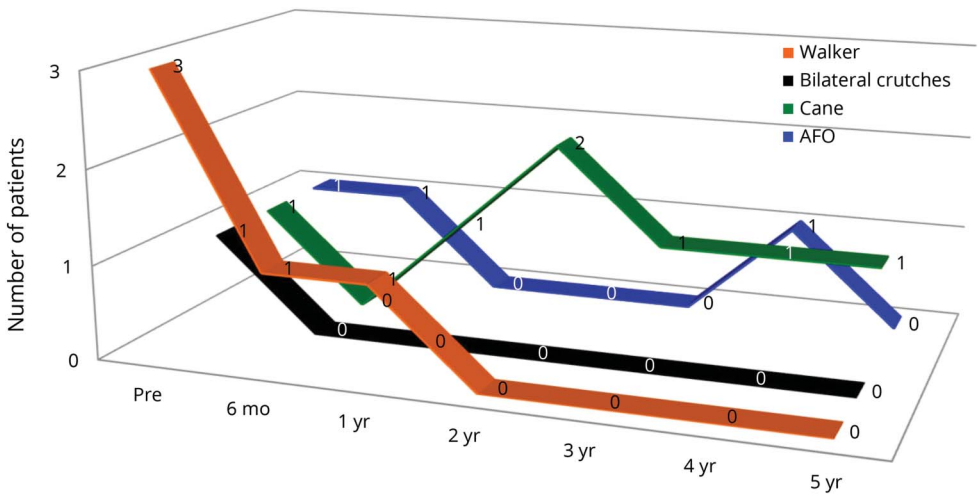
| | Pre-HSCT | 1 y | 5 y |
|---|---|---|---|
| **QOL physical score** | | | |
| **No. of patients**[a] | 11 | 11 | 10 |
| **Median (IQR)** | 24.4 (16.6–38.2) | 41.6 (39.6–64.3) | 52.5 (43–70.95) |
| **Mean (SD)** | 27.95 (16.76) | 50.98 (22.52) | 56.24 (18.16) |
| **95% CI** | 16.69–39.21 | 35.85–66.11 | 43.25–69.23 |
| ***p* Value**[b] | | 0.017 | 0.001 |
| **QOL mental score** | | | |
| **No. of patients** | 11 | 11 | 10 |
| **Median (IQR)** | 40.8 (27.23–50.18) | 63.1 (43.63–73.8) | 69 (53.05–73.95) |
| **Mean (SD)** | 37.64 (16.53) | 59.51 (23.17) | 65.07 (15.99) |
| **95% CI** | 26.53–48.75 | 43.94–75.08 | 53.63–76.51 |
| ***p* Value**[b] | | 0.026 | 0.002 |
| **QOL total score** | | | |
| **No. of patients** | 11 | 11 | 10 |
| **Median (IQR)** | 30.83 (23.15–47.86) | 52.69 (38.75–66.24) | 61.63 (45.84–76.25) |
| **Mean (SD)** | 34.22 (16.57) | 55.08 (22.96) | 62.09 (18.63) |
| **95% CI** | 23.09–45.35 | 39.66–70.5 | 48.76–75.42 |
| ***p* Value**[b] | | 0.032 | 0.001 |

Abbreviations: CI = confidence interval; IQR = interquartile range.
[a] One patient did not complete the pre-HSCT SF-36 and was not included.
[b] Comparison group is before HSCT.

always remained seropositive post-transplant were the only 2 that relapsed after HSCT (table 3 and figure 3) and were restarted on immunosuppressant medications.

Patients who became AQP4-seronegative post HSCT also had a significant drop in their serum complement activating and cell killing ability (figure 4). Serum was

**Figure 2** Device assistance for ambulation before and after hematopoietic stem cell transplantation (HSCT)




The number of patients who required no assistance for ambulation went from 6 of 12 pre-HSCT to 9, 10, 9, 8, 8, and 9 at 6 months, 1, 2, 3, 4, and 5 years post transplantation, respectively. AFO = ankle and foot orthoses.

**Table 3** Commercial neuromyelitis optica antibody assay results and relapse

| Patient | Pre HSCT | 6 mo | 1 y | 2 y | 3 y | 4 y | 5 y | Post HSCT disease status while off all immune suppression drugs |
|---|---|---|---|---|---|---|---|---|
| 1 | Positive (ELISA, FACS) | Negative (IFA), positive (FACS) | Negative (IFA) | — | — | — | Negative (ELISA) | Never relapsed |
| 2 | Positive (ELISA) | Negative (ELISA) | Negative (ELISA) | — | — | — | Negative (CBA, FACS) | Never relapsed |
| 3 | Positive (ELISA, FACS) | Negative (ELISA) | Negative (ELISA) | Negative (ELISA, FACS) | Negative (CBA, FACS) | Negative (CBA, FACS) | Negative (FACS) | Never relapsed |
| 4 | Positive (ELISA) | Positive (ELISA) | Positive (ELISA) | Positive (FACS) | Positive (FACS) | — | Positive (FACS) | Relapsed 2 years after HSCT |
| 5 | Negative (IFA) | Negative (ELISA) | Negative (ELISA) | — | Negative (CBA) | Negative (CBA) | Negative (FACS) | Never relapsed |
| 6 | Positive (IFA, FACS) | — | Negative (ELISA), positive (FACS) | Negative (ELISA), positive (FACS) | — | Positive (CBA, FACS) | Positive (CBA) | Never relapsed |
| 7 | Positive (ELISA, FACS) | Negative (ELISA) | Negative (ELISA), positive (FACS) | Positive (CBA, FACS) | Positive (CBA) | Positive (CBA) | Positive (CBA) | Never relapsed |
| 8 | Positive (ELISA, FACS) | — | Positive (CBA) | — | — | — | No assay but alive, no symptoms | Relapsed 18 months after HSCT |
| 9 | Positive (ELISA, FACS) | Negative (ELISA) | Negative (CBA) | Negative (CBA, FACS) | Negative (CBA, FACS) | Negative (FACS) | Negative (FACS) | Never relapsed |
| 10 | Positive (ELISA, FACS) | Negative (CBA), positive (FACS) | Negative (CBA), positive (FACS) | Positive (CBA, FACS) | | | Negative (FACS) | Never relapsed |
| 11 | Positive (CBA, FACS) | | Negative (IFA) | | | | No assay but alive, no relapse, at 5 years | Never relapsed |
| 12 | Positive (ELISA, FACS) | Negative (FACS) | Negative (FACS) | Negative (FACS) | Not yet reached 3 years | | | Never relapsed |

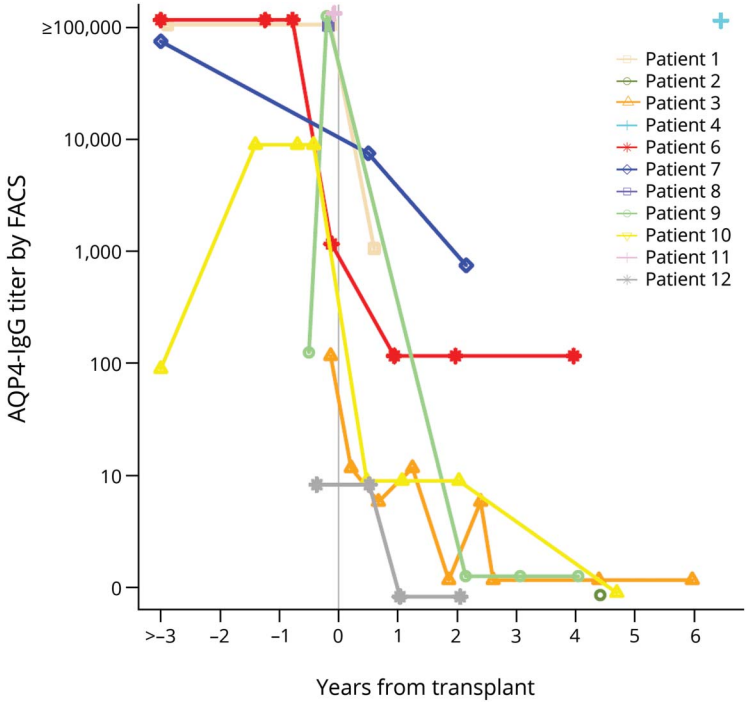
Abbreviations: CBA = cell-binding assay; FACS = fluorescence-activated cell sorting; HSCT = hematopoietic stem cell transplantation; IFA = immunofluorescence assay.
The more sensitive FACS assay was retrospectively performed on stored serum, where available, on patients whose original test was ELISA, IFA, or CBA. Not included is one patient who was aquaporin-4-seropositive with refractory antinuclear antibody–positive systemic lupus erythematosus complicated by glomerulonephritis, nephrotic syndrome, pulmonary hemorrhage, colitis, and neuropsychiatric lupus with seizures and extensive white matter cerebral lesions without longitudinally extensive transverse myelitis or optic chiasm atrophy.

available, post HSCT, in only 1 of 2 patients who relapsed and this patient had the highest AQP4-IgG complement activation titer (end point dilution of 10,240) at 6 years (patient 4, figure 4). Patient 6, who remained AQP4-IgG-positive by FACS (figure 3 and table 3) and relapse-free off immunotherapy at 5 years post HSCT, had a significant drop in complement activation titer (figure 4). Those patients who seroconverted to negative status, and for whom pre and post samples were available for testing, demonstrated a switching off of serum complement activating and cell-killing ability (figure 4) and remained relapse-free.

## Discussion

Autologous HSCT is the commonly used and established terminology for collecting and reinfusing a patient's own hematopoietic stem cells after either immunoablative or myeloablative chemotherapy. The autologous hematopoietic stem cells are a supportive blood transfusion infused to

**Figure 3** Aquaporin-4 (AQP4) titer by AQP4 flow cytometry assay (fluorescence-activated cell sorting [FACS]) pre and post hematopoietic stem cell transplantation (HSCT) from stored serum samples




The color lines represent AQP4–immunoglobulin G (IgG) titers of each patient by FACS assay on stored serum samples. Patients 4 and 8 relapsed after transplantation. Patient 5 was negative pre and post HSCT for both AQP4-IgG index and complement.

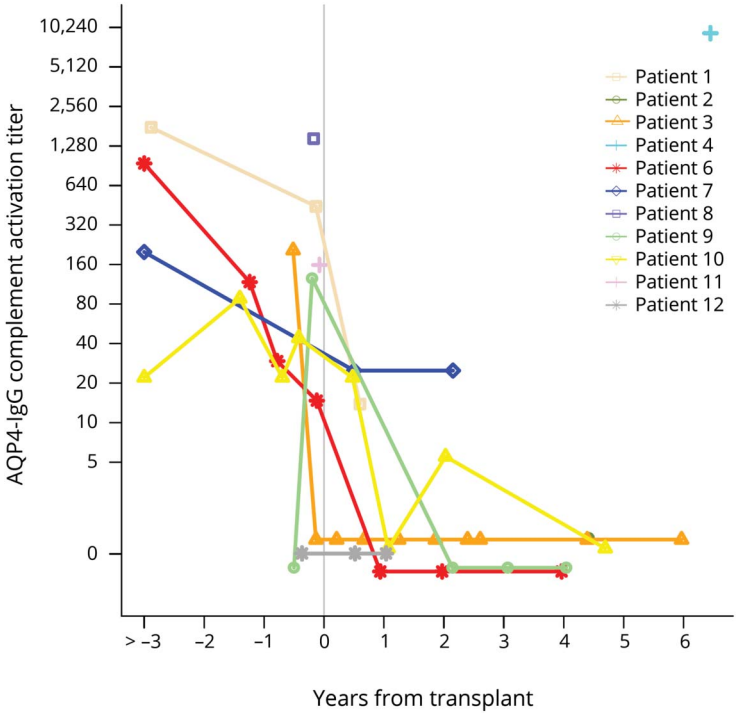
shorten the duration of conditioning regimen-induced cytopenias. Since the toxicity and effect of HSCT arises from the immune suppressive drugs in the conditioning regimen, it would be anticipated that different transplant condition regimens will have different toxicity profiles and different effects on inducing long-term disease remission.

In the pilot study reported herein, all patients received the same nonmyeloablative regimen that included cyclophosphamide, rituximab (anti-B-cell antibody), ATG (antithymocyte antibody), and plasmapheresis. For SLE, a disease associated with autoantibodies, we found, when using an unmanipulated graft, that the addition of rituximab to cyclophosphamide and ATG induced remission in most patients.[29] We therefore theorized that a regimen of rituximab, cyclophosphamide, and ATG could potentially eliminate pathogenic AQP4 antibodies.

The inclusion of rituximab in our conditioning regimen probably contributed to AQP4 seronegative conversion. However, conversion to seronegative status with only rituximab therapy is unlikely.[30–33] Valentino et al.,[30] using a cell-based assay (Euroimmun, Lübeck, Germany), determined AQP4-IgG titers in serial samples from 7 patients receiving rituximab infusions. AQP4-IgG titers dropped in 4, remained unchanged in 2, and increased in 1 (only nonresponder); only 1 of 7 patients seroconverted and that patient had a low/borderline titer at baseline. AQP4-IgG remained detectable in 6 of 7 patients despite undetectable CD19+ B cells in 78% of samples. Kim et al.,[31] using an in-house ELISA methodology, demonstrated that AQP4-IgG titers declined or maintained at low level in the majority of 21 seropositive patients, 2 years after initiation of rituximab. Pellkofer et al.,[32] using an in-house cell-based flow cytometric assay, reported that AQP4-IgG titers did not decline significantly during rituximab treatment in 10 patients. They suggested that since plasma cells do not express CD20, they are not eliminated in the bone marrow and thus the drug had little effect on antibody titers.[32] Jarius et al.,[33] using a fluorescent-based immunoprecipitation assay, reported that AQP4 antibodies were detectable in almost all samples obtained under immunosuppressive therapy. Overall, these studies, though based on relatively small case series and using varied detection methodologies, suggest that AQP4-IgG titers generally drop in the setting of immunotherapy, but rarely seroconvert. Further, we found no publications indicating that any therapy, outside of the current transplant data, prevented complement activation or eliminated cell killing.

Some authors have expressed skepticism towards using HSCT as a therapy for AQP4-IgG-positive NMOSD based on failure to prevent relapse in a single patient treated with a myeloablative cancer regimen for lymphoma.[34] A European Bone Marrow Transplant (EBMT) registry study summarized the outcome of autologous HSCT in 16 patients with NMO.[35] Five-year PFS and RFS were 48% and 10%, respectively, and AQP4-IgG remained positive in the 8 patients evaluated post-transplant. However, the EBMT registry

**Figure 4** Complement activation titers from pre and post hematopoietic stem cell transplantation (HSCT) stored serum samples




The color lines represent aquaporin-4 (AQP4)–immunoglobulin G (IgG) complement activation titers of each patient by fluorescence-activated cell sorting assay on stored serum samples. Patients 4 and 8 relapsed after transplantation. Patient 5 was negative pre and post HSCT for both AQP4-IgG index and complement.

study[35] was a retrospective series of case reports involving multiple different conditioning regimens, none of which included rituximab.

Compared to the EBMT registry data that had a mean pre-transplant EDSS of 6.5 (range 2–8.5),[35] patients in our study had less accumulated disability with a pre-HSCT baseline mean EDSS of 4.3 (range 2–6.5). Patients in our study, unlike the EBMT registry study, also had significant improvements in EDSS, NRS, and quality of life, and post-transplant seroconversion from AQP4-IgG-positive to negative status in the majority of patients. Post-transplant disappearance of the AQP4-IgG antibody also correlated with the inability of a patient's serum to activate complement and kill AQP4-transfected HEK293 cells in vitro.

This pilot study is consistent with the pathogenic role for the AQP4-IgG antibody since all patients who became seronegative improved and never relapsed over a drug-free interval of 5 years (table 3). Furthermore, a significant decrease in their serum complement activating and cell-killing ability was also observed. Patients 6, 7, and 10, who became seronegative after HSCT (table 3), subsequently became seropositive but remained in clinical remission without treatment. Patient 10 converted to seropositive at 2 years but without treatment reverted to seronegative status at 5 years, while in patient 6, although reverting to seropositive, the antibody no longer caused complement activation or cell death (figure 4). Both patients who relapsed always remained seropositive for AQP4-IgG (table 3) and had high complement activation titers before and after HSCT. These data imply that patients who do not become AQP4-IgG-negative within 1 year of autologous HSCT are at an increased risk for relapse and should be monitored closely and may warrant consideration for maintenance immune suppression while those who become AQP4-IgG-seronegative may remain free of immunosuppressive medications over the long run.

Autologous HSCT utilizing a nonmyeloablative regimen that contains cyclophosphamide, rATG, rituximab, and selection of patients who are still able to ambulate, albeit in some cases with a walker, results in neurologic improvements, prolonged relapse-free and treatment-free remissions, and in most cases conversion of AQP4-IgG antibody to seronegative status with inability of the patient's plasma to kill AQP4-transfected cells in vitro. Further studies will be needed to confirm these results in AQP4-IgG-positive NMOSD.

## Author contributions

R.K. Burt: drafting/revising the manuscript, data acquisition, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval. R. Balabanov: drafting/revising the manuscript, data acquisition, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, study supervision. X. Han: drafting/revising the manuscript, data acquisition, analysis or interpretation of data, accepts responsibility for conduct of research and final

approval. C. Burns: data acquisition, accepts responsibility for conduct of research and final approval. J. Gastala: data acquisition, accepts responsibility for conduct of research and final approval. B. Jovanovic: study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, statistical analysis. I. Helenowski: analysis or interpretation of data, accepts responsibility for conduct of research and final approval, statistical analysis. J. Jitprapaikulsan: drafting/revising the manuscript, analysis or interpretation of data, accepts responsibility for conduct of research and final approval, acquisition of data. J.P. Fryer: drafting/revising the manuscript, accepts responsibility for conduct of research and final approval, acquisition of data. S.J. Pittock: drafting/revising the manuscript, study concept or design, analysis or interpretation of data, accepts responsibility for conduct of research and final approval.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Richard K. Burt, Roumen Balabanov, Xiaoqiang Han, et al.

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