Effects of Age and Sex on Aquaporin-4 Autoimmunity

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Objective: To determine the sex and age distribution of aquaporin-4 (AQP4) autoimmunity using data derived from clinical service laboratory testing of 56,464 patient samples.

Design: Observational analysis.

Setting: Mayo Clinic Neuroimmunology Laboratory.

Patients: Between October 1, 2005, and January 4, 2011, 56,464 patients were tested for AQP4-IgG; 2960 (5.2%) patients were seropositive.

Main Outcome Measure: Seropositivity for AQP4-IgG.

Results: Patients seropositive for AQP4-IgG were older than seronegative patients (mean [SD] age, 46 [16] vs 42 [15] years, respectively; P < .001). More females than males were tested (37,662 vs 16,810, respectively; P < .001). Among 2743 seropositive patients, 146 (5.3%) were pediatric (aged ≤18 years) and 333 (12.1%) were elderly (aged ≥65 years). The sex distribution of seropositive patients was 2465 females and 306 males (absolute female:male ratio, 8.1:1; P < .001). After adjusting for the number of females tested, an excess of females persisted (adjusted female:male ratio, 3.6:1). Female predominance for AQP4-IgG was more striking in adults (absolute female:male ratio, 8.4:1; adjusted female:male ratio, 3.5:1) than in pediatric patients (absolute female: male ratio, 4.3:1; adjusted female: male ratio, 2.9:1) (P < .001). Elderly women were more likely to be seropositive than individuals in other age categories (13.1% vs 6.0%, respectively; P < .001). The proportion of AQP4-IgG-seropositive individuals (detection rate), defined by decade of age, increased exponentially in women after age 50 years.

Conclusions: Seropositivity for AQP4-IgG occurs predominantly in females, particularly in individuals older than 18 years. Among seropositive patients, 1 in 6 is in the extremes of age. The detection rate of AQP4-IgG increased in women after age 50 years.


NEUROMYELITIS OPTICA (NMO) is an inflammatory autoimmune demyelinating disease of the central nervous system characterized by relapsing optic neuritis and transverse myelitis and may cause blindness and paraplegia.1 It is frequently misdiagnosed as multiple sclerosis. It is the first inflammatory demyelinating central nervous system disorder for which a specific target antigen, the astrocytic water channel aquaporin-4 (AQP4), has been identified.2 The AQP4-specific autoantibody (NMO-IgG) is a clinically validated serum biomarker that distinguishes relapsing NMO and its partial or inaugural forms (NMO spectrum disorders) from multiple sclerosis.3,4 Epidemiological data unanimously indicate that women are more prone to develop NMO, but the magnitude of the female prevalence varies considerably between studies.5 Studies using clinical diagnostic criteria to identify patients with NMO1,5 tend to report lower female: male ratios (range, 2.4-4.1:1).6-10 while studies identifying individuals on the basis of AQP4-IgG seropositivity report significantly higher female: male ratios (range 7-12.1:1).11-13

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Table. Although children13,14 and elderly individuals15 are recognized to be vulnerable to developing NMO, study of sex differences among patients of different ages has been limited by inadequately powered sample sizes.

From 2004 until 2011, the Mayo Clinic Neuroimmunology Laboratory served as the only clinical reference center for AQP4-IgG evaluation in the United States. The aim of this study is to determine the
sex and age distribution of AQP4 autoimmunity using data derived from AQP4-IgG testing in this laboratory.

### METHODS

Between October 1, 2005 (formal date of service testing implementation), and January 4, 2011, 56 464 serum or cerebrospinal fluid specimens were tested for AQP4-IgG. Only 1 specimen was counted for patients with more than 1 specimen tested (whichever was first positive) and the age recorded was when AQP4-IgG was first detected. Seropositivity for AQP4-IgG was detected in 2960 patients (5.2%). Information on sex and age was available from the test request form for 2771 and 2743 patients, respectively. For the 53 504 patients whose results were detected in 2960 patients (5.2%). Information on sex and age was available from the test request form for 2771 and 2743 patients, respectively. For the 53 504 patients whose results were derived from AQP4-IgG testing in this laboratory.

### RESULTS

The mean (SD) age of individuals for whom AQP4-IgG testing was requested was 42.3 (14.9) years (range, 1-99 years); testing was requested more frequently for females than males (37 662 females vs 16 810 males; sex ratio, 2.2:1; P < .001). Seropositive patients were older than seronegative patients (mean [SD] age, 46 [16] vs 42 [15] years, respectively; P < .001).

**FEMALE PREDOMINANCE IN AQP4 AUTOIMMUNITY**

Of 2771 seropositive patients, 2465 were female and 306 were male (absolute female: male ratio, 8.1:1; P < .001). Subgroup analysis of Mayo Clinic seropositive patients revealed a comparable female-predominant absolute female: male ratio (6.9:1). Of the 51 701 seronegative patients, 35 197 were female and 16 504 were male (absolute female: male ratio, 2.1:1; P < .001). Corrections were performed to take into account the disproportionate number of females tested. After these adjustments, the female predominance persisted (female: male ratio, 3.6:1).

**FEMALE PREDOMINANCE HIGHEST IN ELDERLY PATIENTS AND LOWEST IN CHILDREN**

To investigate the sex differences for seropositive patients according to age, we stratified individuals to 4 age groups (Figure 1A and B): pediatric (≤18 years), young adult (19-39 years), middle-aged adult (40-64 years), and elderly (≥65 years). Female predominance was more striking in adult patients (aged ≥19 years; absolute female: male ratio, 8.1:1; P < .001). Corrections were performed to take into account the disproportionate number of females tested. After these adjustments, the female predominance persisted (female: male ratio, 3.6:1; P < .001).
male ratio, 8.4:1; female:male ratio adjusted for bias in test request frequency, 3.5:1) compared with pediatric patients (absolute female:male ratio, 4.3:1; adjusted female:male ratio, 2.9:1) ($P < .001$). Subgroup analysis of Mayo Clinic seropositive patients revealed similar sex differences stratified by age when compared with the primary cohort.

**Figure 1.** Female preponderance and extremes of age of aquaporin-4 autoimmunity. A, The female:male ratios of 2771 patients testing positive for aquaporin-4–IgG are compared in 4 age groups: pediatric (<18 years), young adult (19-39 years), middle-aged adult (40-64 years), and elderly (≥65 years). The absolute female:male sex ratios of seropositive patients are 4.3:1 in the pediatric group, 9.2:1 in young adults, 8.1:1 in middle-aged adults, and 8.2:1 in the elderly group. B, The corrected sex ratios of seropositive patients are 2.9:1 in the pediatric group, 3.6:1 in young adults, 3.5:1 in middle-aged adults, and 4.9:1 in the elderly group. C, The age distribution of 2743 patients testing positive for aquaporin-4–IgG is shown. Subgroup analysis findings of the Mayo Clinic cohort are comparable.

**Figure 2.** Analysis of detection rates (proportion of seropositive individuals defined by decade of age) of aquaporin-4 (AQP4)–IgG in females and males revealed a higher detection rate for females compared with males. An increased likelihood of detecting AQP4 autoimmunity was found in females after age 50 years, with the female detection rate increasing exponentially from ages 51 to 60 years.

**EXTREMES OF AGE ACCOUNT FOR 1 IN 6 PATIENTS WITH AQP4 AUTOIMMUNITY**

Pediatric and elderly patients accounted for 12.6% of those tested. Of the 2743 seropositive patients, 146 (5.3%) were pediatric and 333 (12.1%) were elderly. Thus, approximately 1 in 6 AQP4-IgG–seropositive patients were at the extremes of age. Findings were comparable for the Mayo Clinic cohort (Figure 1C).

**INCREASED DETECTION RATE OF AQP4-IgG IN WOMEN OLDER THAN 50 YEARS**

Women in the elderly group were more likely to test positive than those in other age categories (13.1% vs 6.0%, respectively; $P < .001$). A more detailed analysis of detection rates (proportion of seropositive individuals defined by decade of age) revealed an exponential increase in the AQP4-IgG detection rate after age 50 years, especially for women (Figure 2).

**COMMENT**

This seroepidemiologic study of AQP4-IgG status among 56 464 patients tested on a service basis reports several important new observations: (1) females are more likely to be seropositive than males, even after correcting for excess testing in females, and this female predominance is more striking in adults than in children; (2) one-sixth of AQP4-IgG seropositivity was detected in children and elderly patients; and (3) the detection rate of AQP4-IgG increases exponentially in women older than 50 years.

Previous studies of female:male ratios in patients with NMO have reported values ranging from 2.4:1 to 12.1:1. These inconsistencies are likely explained by differences in 1 or more of the following: patient acquisition (clinically acquired vs referral for serological evaluation), regional or international patient referral patterns (to either general neurology or demyelinating disease clinic-
ics and testing laboratories), and disease biology (among AQP4-IgG-seropositive and AQP4-IgG-seronegative patients). Some clinic-based studies have reported the proportion of AQP4-IgG-seropositive patients but do not mention sex ratios among seropositive and seronegative patients.\textsuperscript{7,10} We recently reported a clinic-based study of 99 consecutive Mayo Clinic patients with NMO treated with azathioprine; the female: male ratios in that cohort were 4:1 in all 93 AQP4-IgG-tested patients, 6:1 in AQP4-IgG-seropositive patients, and 2:1 in AQP4-IgG-seronegative patients.\textsuperscript{17} Thus, while a female: male ratio of 8:1:1 in the current study may be an overestimate, current evidence points to the female preponderance being stronger among cohorts of seropositive patients than among clinically acquired cohorts and seronegative patients. Future population-based serological studies of demyelinating disorders will add further to our understanding.

Although the evidence for humoral immune mechanisms underlying the pathogenesis of NMO is compelling at this stage, the etiology remains unknown. Genetic susceptibility,\textsuperscript{15} infections,\textsuperscript{19} and cancer associations\textsuperscript{20} have been implicated. Our observation of an exponentially increasing AQP4-IgG detection rate in elderly women (aged >50 years) compared with elderly men suggests that aging and/or sex hormones may influence the development of AQP4 autoimmunity and its associated spectrum of inflammatory central nervous system disorders. Furthermore, the female predominance was greatest in elderly patients and least in children. These findings are in contrast with those by Nagaishi et al.,\textsuperscript{15} which suggested a decreasing female predominance with age. It is unclear whether the small sample size of the latter study, which comprised 9 patients younger than 15 years and 60 patients older than 60 years, could have led to erroneous representations of sex differences in different age groups.

In a disease typically regarded as disproportionately affecting middle-aged patients, we found that approximately one-sixth were at the extremes of age (pediatric and elderly). These findings agree with observations reported in a French study:\textsuperscript{12} 25% of patients with NMO had disease onset before age 19 years or later than age 50 years. The heterogeneous cerebral presentations of AQP4 autoimmunity in the pediatric group\textsuperscript{15} underscore the importance of serological testing to aid clinical distinction from acute disseminated encephalomyelitis. The high rate of AQP4-IgG detection in elderly patients, particularly women, is also noteworthy. Although aging and menopause could contribute to immune dysfunction leading to AQP4 autoimmunity, it remains possible that this observation may relate to different clinical thresholds for AQP4-IgG testing applied for different age groups, i.e., lower threshold for testing in younger individuals compared with elderly individuals. These findings emphasize the importance of considering NMO spectrum disorders in the differential diagnosis of a new-onset central nervous system disorder occurring at the extremes of age.

Our study has several limitations. The study population comprises patients with heterogeneous clinical manifestations for which AQP4-IgG testing was requested. Details of these manifestations, however, were not available for this analysis except for the Mayo Clinic patients. Furthermore, some seropositive cases may have been missed owing to limited assay sensitivities or immunosuppressive therapy reducing antibody levels below the assay detection threshold. Notwithstanding, this study, which reports observations from the only clinical laboratory in the United States performing this antibody test on a service basis until 2011, raises pertinent questions relating to the influence of sex hormones and aging on AQP4 autoimmunity. The cross-sectional study design does not provide information on disease onset and its evolution. Future prospective studies are warranted to investigate their contributions to production of AQP4-IgG and clinical manifestations of NMO spectrum disorders.
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**REFERENCES**