Short Report

Incidence of AQP4-IgG seropositive neuromyelitis optica spectrum disorders in the Netherlands: About one in a million

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Abstract

Neuromyelitis optica (NMO) is a rare autoimmune disease affecting the optic nerves and spinal cord. In the majority of NMO patients anti-aquaporin-4 antibodies (AQP4-IgG) are detected. Here we assessed a nationwide incidence of AQP4-IgG-seropositive NMO spectrum disorders (NMOSD) in the Netherlands based on results of one central laboratory. Data were collected since the introduction of the highly sensitive cell-based assay for six consecutive years. Samples from 2795 individual patients have been received; of them 94 (3.4%) were seropositive. Based on the Dutch population with 16.6 million inhabitants, the mean incidence of AQP4-IgG-seropositive NMOSD was calculated at 0.09 per 100,000 people.

Introduction

Neuromyelitis optica (NMO) is a rare autoimmune disease classically affecting the optic nerves and spinal cord.[1] Exact incidence figures of NMO in the Netherlands are currently unknown. The clinical spectrum of NMO has broadened in the past years and in addition to Devic's syndrome it includes limited forms such as isolated or recurrent optic neuritis, transverse myelitis, brainstem syndromes and other cerebral presentations.[2,3] In approximately 77% of the patients with NMO spectrum disorders (NMOSD), specific antibodies against aquaporin-4 (AQP4-IgG) are detected.[2] In the Netherlands diagnostic testing of these antibodies is performed in one centralised NMO expert centre. This provides a unique chance to gain insight into the nationwide incidence of AQP4-IgG-seropositive NMOSD. Epidemiological figures of NMOSD are of interest for patient care and counselling and for the estimation of the socioeconomic burden of the disease. The purpose of this study is to estimate the nationwide incidence of NMOSD in the Netherlands.

Methods

Patients

This study was conducted at the Dutch national NMO expert centre, which includes Sanquin Diagnostic Services in Amsterdam and the NMO expert clinic at the Erasmus University Medical Centre (Erasmus MC) in Rotterdam. We collected demographic data (age and gender) from serum samples sent for routine AQP4-IgG diagnostics. Data were collected since the nationwide availability of the highly sensitive cell-based assay (CBA) for AQP4-IgG detection in May 2009, and the period of observation included the following six consecutive years. Samples sent in from abroad, mainly Belgium and the Dutch Caribbean, were excluded from this study ($n = 139$ patients). Of these foreign patients, eight were AQP4-IgG seropositive. Incidence rates were calculated as the number of AQP4-IgG-seropositive patients per year divided by the number of Dutch inhabitants per 100,000 people. Population figures were extracted from Statistics Netherlands.[4] From the patients known at the Erasmus MC in Rotterdam clinical data were collected. Magnetic resonance images (MRIs) were evaluated for the presence of lesions, longitudinally extensive transverse myelitis (LETM)[3] and cerebral NMO-like lesions.[5] In five patients the diagnosis of NMOSD could have been made prior to the time of the AQP4-IgG assay in 2009 based on their clinical characteristics and therefore they were not included in the incidence calculations. This study was

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Table 1. Incidence rates of six consecutive years of AQP4-IgG-seropositive NMOSD in the Netherlands. Population figures were extracted from Statistics Netherlands.[4]

Year	Number of AQP4-IgG-seropositive NMOSD patients	Number of Dutch inhabitants	Incidence per 100.000 people
1: May 2009–April 2010	15	16,486,000	0.09
2: May 2010–April 2011	15	16,575,000	0.09
3: May 2011–April 2012	12	16,656,000	0.07
4: May 2012–April 2013	16	16,730,000	0.10
5: May 2013–April 2014	18	16,778,000	0.11
6: May 2014–April 2015	13	16,829,000	0.08
Mean/year	**15[a]**	**16,676,000**	**0.09**

[a]Results rounded to the nearest integer.
AQP4-IgG: aquaporin-4 immunoglobulin G; NMOSD: neuromyelitis optica spectrum disorders.

approved by the Medical Ethical Committee of the Erasmus MC in Rotterdam. All patients from the Erasmus MC provided informed consent.

AQP4-IgG CBA

We used a CBA for AQP4-IgG detection as has previously been described.[6] In short, patient serum was incubated with HEK293 cells transiently transfected with AQP4-M23 (final serum dilution 1:20). After washing, cells were subsequently incubated with goat anti-human IgG Allophycocyanin (APC)-conjugated secondary antibody and analysed after washing using fluorescence-activated cell sorter (FACS). The cutoff was determined in every assay as average deltaMFI + 10 standard deviations of eight individual negative control sera.

Statistical analysis

Statistical analysis was performed using SPSS 21.0. The Chi-Square test and Mann-Whitney U test were used to compare categorical and continuous data, respectively.

Results

During six consecutive years, from May 2009 until May 2015, 3207 samples of 2795 individual Dutch patients were received for AQP4-IgG testing. Samples were sent from 85 different hospitals including all eight university hospitals. Of all included patients, 94 (3.4%) were seropositive. A total of 240 children and adolescents younger than 18 years old were included; of them, eight (3.3%) were AQP4-IgG seropositive. The mean age of AQP4-IgG-seropositive patients was 47.6 years ± 18.2 compared with 41.0 years ± 16.1 in the seronegative group ($p < 0.01$). Seventy-eight (83%) of the seropositive patients were female in contrast to 1698 (63%) female patients in the seronegative group ($p < 0.01$). The incidence rates of six consecutive years are presented in Table 1. The mean incidence of NMOSD during the past six years in the Netherlands was calculated at 0.09 per 100,000 people. Considering that approximately 77% of NMOSD patients have antibodies directed to AQP4,[2] the estimated incidence of NMOSD in general (including AQP4-IgG-seropositive and -seronegative cases) is 0.12 per 100,000 people. Thirty-six of the 94 AQP4-IgG-seropositive NMOSD patients (38%) are known at the Erasmus MC and their clinical data are presented in Table 2. Seventy-eight per cent of them were females. Twenty-four patients had LETM at some point during their disease course. Eventually at last follow-up 21 patients (58%) fulfilled classic NMO criteria with optic neuritis and transverse myelitis.[3]

Discussion

Here we report the incidence of AQP4-IgG-seropositive NMOSD in the Netherlands, derived from data of the Dutch national NMO expert centre, as nearly one in a million: 0.09 per 100,000 people. Unique to this study is that we have nationwide coverage given that the CBA is performed in one central laboratory. Our incidence figure is within the range of previously described incidence rates which range from 0.05–0.4 per 100,000 people.[7] It has to be considered that epidemiological studies on NMOSD are difficult to compare since they are based on different selection and inclusion criteria. For example, different clinical definitions and AQP4-IgG assays were used. Also the ethnicities of included patients and the geographic coverage differed. Two studies performed in

Table 2. Clinical characteristics of 36 AQP4-IgG-seropositive NMOSD patients known at the Erasmus MC.

	AQP4-IgG seropositive NMOSD patients, $n = 36$
Age at onset, mean years (SD)	41.6 (18.9)
Females, *n* (%)	28 (78%)
Caucasians, *n* (%)	27 (75%)
AID comorbidity, *n* (%)	8 (22%)
Time from first onset of symptoms to APQ4-IgG assay, median months (range)	7.9 (0.3–248.8[a])
Type of onset, *n* (%)	
ON	12 (33%)
TM	18 (50%)
NMO	4 (11%)
Brainstem and or cerebral syndromes	2 (6%)
CSF elevated IgG index >0.68 and/or positive OCB, *n* (%)	11/31 (35%)
MRI cerebral lesions, *n* (%)[b]	17/34 (50%)
NMO-like[5]	4 (12%)
Aspecific	13 (41%)
MRI spinal cord lesions, *n* (%)[b]	30/33 (91%)
LETM	24 (73%)
Relapse, *n* (%)	24 (67%)
Chronic treatment, *n* (%)	30 (83%)
Follow-up, mean years (SD)	5.4 (5.4)
Type at last follow-up, *n* (%)	
ON	3 (8%)
TM	11 (31%)
NMO	21 (58%)
Brainstem and or cerebral syndromes	1 (3%)

[a]The extreme of 248.8 months from onset to sampling was caused by a patient with recurrent optic neuritis in 1988, 2004 and later. In this particular case NMOSD diagnosis could not have been made prior to the AQP4-IgG testing. [b]MRIs performed at onset and/or follow-up.
AID: autoimmune disease; AQP4-IgG: aquaporin-4 immunoglobulin G; CSF: cerebrospinal fluid: IgG: immunoglobulin G; LETM: longitudinally extensive transverse myelitis; NMO(SD): neuromyelitis optica (spectrum disorders); OCB: oligoclonal bands; ON: optic neuritis; TM: transverse myelitis; MRI: magnetic resonance imaging.

comparable geographic areas in Denmark and the United Kingdom differed essentially from our study, as both studies also included AQP4-IgG-seronegative NMOSD patients and did not have nationwide coverage.[8,9]

A comparable Austrian study calculated an incidence of 0.05 per 100,000 people.[10] The main difference with our study is that the identified patients were all Caucasian. However there are indications that some ethnic groups are overrepresented in NMOSD.[11] In the Netherlands we estimated the incidence of NMOSD is more than twice as high in non-Caucasians. Based on 25 per cent of the patients

known at Erasmus MC were non-Caucasian and 11.9 per cent of the Dutch inhabitants are non-Caucasian[4] we estimated a mean annual incidence rate of NMOSD for non-Caucasians of 0.19 per 100,000 people and for Caucasians of 0.08 per 100,000 people.

We think our findings reflect the real incidence of AQP4-IgG-seropositive NMOSD in the Netherlands. However, we cannot exclude that mild cases and forme fruste types of the disease[2] have been missed. Fifty-eight per cent of the NMOSD patients at the Erasmus MC fulfilled classic NMO criteria.[3] Unfortunately we did not have access to the clinical data of all patients and therefore we could not present

this figure for all NMOSD patients in the Netherlands. Only the clinical data of patients known at the Erasmus MC are presented; these, however, cover more than one-third of the study population.

More awareness and better recognition of NMOSD might increase the incidence in the future. Further demographic studies and international collaboration in the NMO field would add to a better understanding of NMOSD.

Conflict of interest
None declared.

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