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EPIDEMIOLOGY OF AUTOIMMUNE NEUROLOGIC DISORDERS IN SARDINIA
Comparison between NMOSD and Myasthenia Gravis

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4) DISCUSSION AND CONCLUSIONS

ABSTRACT

Over the last two decades, the spectrum of neurologic disorders associated with specific autoantibody biomarkers have expanded. These autoimmune disorders may affect both the central and peripheral nervous system, including the neuromuscular junction. Aquapoin-4-IgG-positive neuromyelitis optica spectrum disorder (AQP4-IgG+NMOSD) and myasthenia gravis (MG) are among the most common autoimmune neurologic disorders of the central and peripheral nervous system, respectively. Although epidemiological studies are crucial to better understand the burden of these rare diseases in the population and adequately plan healthcare assistance, data on incidence and prevalence of autoimmune neurologic disorders in Italy are scarce and outdated. Among Italian regions, Sardinia has historically been studied for its unique characteristics with an unusually high prevalence of different immune-mediated disorders, including multiple sclerosis; but data on antibody mediated neurologic disorders are lacking. The aim of this thesis was to investigate the incidence and prevalence of AQP4-IgG+NMOSD and MG in the island of Sardinia. We found that, while the incidence and prevalence of AQP4-IgG+NMOSD is in line with that of other countries with a predominantly White population, the incidence and prevalence of MG in Sardinia are disproportionally high compared to what previously reported in the literature. In particular, the prevalence of MG in Sardinia seems to exceed the established European threshold to define a rare disorder. Our data represent the first step for an extensive assessment and comparison of the epidemiology of different autoimmune neurologic disorders in Sardinia, which might help better understand the risk factors associated with each disorder and help planning healthcare resources and treatment strategies.

1) INTROUCTION

The expanding spectrum of autoimmune neurologic disorders

Autoimmune neurologic disorders are a group of disorders of the central and/or peripheral nervous system where a specific autoantibody biomarker directed against neural (neuronal or glial) targets can be detected in serum and/or cerebrospinal fluid (CSF). Some of these diseases may have a strong association with cancer, and are known as paraneoplastic neurologic syndomes.[1]

Over the last 40 years, the number of neural autoantibodies associated with neurologic syndromes has grown dramatically, and new antibody specificities are identified every year.[2, 3] This is mainly due to major refinements in the assays for autoantibody detection and discovery (*e.g.,* cell-based assay), and better clinical-MRI characterization of different neurological syndromes.[3] Neural autoantibodies are generally classified based on the cellular location of their target antigens.[4] In particular, antibodies directed against antigens that are expressed on the cell surface are thought to be directly pathogenetic as they can interacting directly with their target. The syndromes associated with these autoantibodies generally show a good response to immunosuppressive therapy, affect a broader age range involving both children and adults, and have a weak paraneoplastic association. On the other hand, antibodies directed against intracellular targets located in the cell nucleus or cytoplasm, and thus not directly accessible by the antibody, are thought to be non-pathogenetic. Neurologic damage would be due to direct cell-mediated cytotoxicity, while the autoantibodies produced during the immune response would only serve as a diagnostic biomarker. Neurologic syndromes associated with antibodies targeting intracellular antigens are often paraneoplastic, respond poorly to immunosuppressive therapy, and are rarely seen in children.[5, 6]

Neurologic syndromes typically associated with neural antibodies are limbic encephalitis or other types of encephalitis, cerebellar ataxia, acute disseminated encephalomyelitis (ADEM), longitudinally extensive myelitis, optic neuritis, stiff-person spectrum disorders, myasthenia gravis, and certain neuropathies.[1] **Table 1** and **Table 2** summarize the main neural autoantibodies identified so far, with commonly associated syndromes, frequency of cancer association, and most affected age range. Notably, immune-mediated neurologic disorders that are not associated with a specific autoantibody biomarker (*e.g.*, multiple sclerosis) are not included among autoimmune neurologic disorders.

NMOSD and Myasthenia gravis

Neuromyelitis optica spectrum disorders (NMOSD) and myasthenia gravis (MG) are among the most common and best characterize antibody-mediated disorders of the central and peripheral nervous system, respectively.

NMOSD is typically associated with antibodies directed against the aquaporin-4 water-channel (AQP4-IgG), although seronegative forms can be diagnosed if strict clinical-radiologic criteria are met.[7, 8] However, recent data suggest that patients diagnosed with seronegative NMOSD have a different disease pathophysiology and yet unidentified antibodies different from AQP4-IgG are likely to play a role in these cases.[9] In this work, the term NMOSD will refer to the AQP4-IgG-positive forms, unless otherwise specified. NMOSD is clinically characterized by recurrent attacks of myelitis (typically longitudinally extensive for more than 3 contiguous vertebral body segments), optic neuritis and/or brain/brainstem dysfunction.[7] The most common brain/brainstem syndrome in these patients is the area postrema syndrome, characterized by intractable nausea, vomiting and hiccups. Disease attacks are generally severe and may result in

major disability if not controlled over time, with an attack-related or stepwise disability accumulation observed in these patients, while a progressive disease course is rare.

For many years, the neuromyelitis optica syndrome was considered by many a severe variant of multiple sclerosis (MS), until AQP4-IgG was discovered in 2004-2005, allowing for the first time to differentiate etiologically another demyelinating central nervous system (CNS) disorder from MS.[10, 11] More recently, the discovery of specific antibodies directed against the MOG protein has allowed to delineate a second autoimmune demyelinating CNS disorder termed MOG-IgG-associated disease (MOGAD).[12, 13] Although together NMOSD and MOGAD are 60-80 times less common than MS among patients with new-onset demyelinating CNS disorders, their identification and early diagnosis is crucial as treatment often differs from that of MS and certain drugs that are effective for MS may worsen NMOSD and MOGAD.[7]

MG was the first autoimmune neurologic disorder to be characterized and represents one of the best understood example of antibody-mediated damage in neurology.[14] Patients present autoantibodies directed against the acetylcholine receptor (AChR-IgG) in 85% of cases; while 5% of patients exhibit antibodies directed against a muscle-specific kinase (MuSK-IgG). A minority of patients with symptoms suggestive of MG remain seronegative for both AChR-IgG and MuSK-IgG; in these patients, other less common or yet unidentified antibody specificities are likely to play a role.

Clinically, the presence of these autoantibodies results in dysfunction of the neuromuscular junction that results in abnormal muscle fatigability and weakness. Symptoms may be restricted to certain muscle groups, like in the case of ocular muscles in ocular MG, or affect diffusely more muscle groups (generalized MG). Approximately 15% of patients have a thymoma, while many have thymic hyperplasia. MG is definitely

the most common autoimmune neurologic disorder and represents an important cause of disability and healthcare expenses in the World.

The Italian Island of Sardinia

The Italian Region of Sardinia is an island of 24,090 km^2 in the Mediterranean Sea, and the second largest in Italy after Sicily. A population of 1,628,384 inhabitants was recorded in 2020, mostly of White ethnicity. The average age of the Sardinian population I significantly older compared to the World, due to its longevity and tendency to emigrate of young people, mostly seeking for better job opportunities. Due to its long history and isolated geographical location, the Sardinian population has been demonstrated to be genetically different from the rest of the Italian population.[15, 16]

Sardinia has been long recognized for its unique epidemiologic characteristics. In particular, previous studies have documented an increased prevalence of certain immune-mediated disorders, including type I diabetes and autoimmune thyroiditis.[17-19] In neurology, Sardinia has extensively been studied for its disproportionally high prevalence of MS, which remains among the highest in the World.[20-23] Epidemiological data on autoimmune neurologic disorders in Sardinia are scarce and outdated.

Aims of the project

The specific aims of this project were:

- To determine the epidemiology of NMOSD in Sardinia
- To determine the epidemiology of seropositive MG in the District of Sassari (Sardinia)

Assessing the epidemiology of two of the most common autoimmune neurologic disorders in Sardinia and make a comparison with that of other neurologic disorders in

the island might help understand disease determinants and guide allocation of healthcare resources.

Table 1 - Demographic and clinical characteristics of the main autoantibodies targeting cell-surface antigens associated with neurologic syndromes according to the largest series reported

Cell-surface target	Female sex, %	Age range, years	Associated syndromes	Cancer association (cancer type)
AChR[24]	40-50%	>50	Myasthenia gravis	15% (thymoma)
AMPAR[25, 26]	65-90%	60-70	LE; hyponatremia	64% (small-cell lung)
AQP4	70-90%	30-40	Optic neuritis; myelitis; area postrema syndrome; encephalopathy/focal brain symptoms (uncommon)	Rare
CASPR2[27, 28]	10-25%	60-70	Encephalopathy; Morvan's/Isaac's syndrome; ataxia	10-20% (thymoma)
DPPX[29, 30]	10-40%	50-60	GI symptoms (diarrhea, episodic severe weight loss); sleep disturbances	10% (hematologic malignancies)
$GABA_AR$[31]	50%	40-50	Seizures/status epilepticus; movement disorders	40% (thymoma)
$GABA_BR$[32-34]	40-65%	60-70	LE; Status epilepticus	50% (small cell lung)
mGluR5[35]	45%	20-30	LE; Viral-like prodromes; seizures	64% (Hodgkin's lymphoma)
GlyRα1[36, 37]	45%	40-50	SPS; PERM	10% (thymoma, seminoma)
IgLON5[38, 39]	50%	60-70	Sleep disturbances; Bulbar symptoms; ataxia	Rare
LGI1[27, 40]	35-40%	60-70	LE; FBDS; hyponatremia	1-10% (thymoma)
MOG[41-43]	50-70%	30-40	ADEM; ON; myelitis; brainstem symptoms; viral-like prodromes	Rare
MuSK[44]	70-90%	30-40	Myasthenia gravis	10-15% (varied)
Neurexin 3α[45]	80%	40-50	Encephalitis; Viral-like prodrome; oro-facial dyskinesia; central hypoventilation; positive ANA	Unknown
NMDAR[46, 47]	80-90%	20-30	LE; psychosis; viral-like prodrome; dyskinesias; central hypoventilation	40-60% (teratoma, usually ovarian)

Abbreviations: AChR = acetylcholine receptor; ADEM = acute disseminated encephalomyelitis; AMPAR = α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ANA, anti-nuclear antibody; CASPR2 = contactin associated protein 2; DPPX = dipeptidyl aminopeptidase-like protein 6; FBDS = facio-brachial dystonic seizures; $GABA_AR$ = γ-aminobutyric acid type-A receptor; $GABA_BR$ = γ-aminobutyric acid type-B receptor; GI = gastrointestinal; mGluR5 = metabotropic glutamate receptor 5; GlyRα1 = glycine receptor subunit alpha-1; IgLON5 = immunoglobulin-like cell adhesion molecule IgLON family member 5; LE = limbic encephalitis; LGI1 = leucine-rich glioma inactivated 1; MuSK = muscle-specific kinase; MOG = myelin oligodendrocyte glycoprotein; NMDAR = N-methyl-D-aspartate receptor; ON = optic

neuritis; PERM = progressive encephalopathy with rigidity and myoclonus; SPS = stiff-person syndrome. [Modified from Table 1 in: Sechi E, Flanagan EP. Curr Treat Options Neurol. 2019 Feb 27;21(3):11].

Table 2 - Demographic and clinical characteristics of the main autoantibodies targeting intracellular antigens associated with neurologic syndromes according to the largest series reported

Intracellular target	Female sex, %	Age range, years	Associated syndromes	Cancer association (cancer type)
AK5[48]	30%	60-70	LE	0%
AGNA (SOX1)[49]	50%	60-70	Lambert-Eaton myasthenic syndrome	>80% (small cell lung)
Amphiphysin[50]	60%	60-70	Encephalopathy; Peripheral neuropathy; myelitis; SPS	80% (breast, small cell lung)
ANNA-1 (Hu)[51, 52]	55-65%	60-70	LE; sensory neuronopathy; autoimmune GI dysmotility	85-90% (small cell lung)
ANNA-2 (Ri)[53]	65%	60-70	brainstem symptoms; opsoclonus-myoclonus; laryngospasm; jaw opening dystonia; ataxia	75% (small cell lung, breast)
ANNA-3[54]	50-60%	50-60	Limbic encephalitis; cerebellar ataxia; peripheral neuropathy; myelopathy	80-90% (small cell lung)
CRMP5 (CV2)[55, 56]	30-60%	60-70	chorea; optic neuropathy/retinopathy; peripheral neuropathy; myelitis	90% (small cell lung, thymoma)
GAD-65[57, 58]	75-85%	50-60	LE; SPS; ataxia; seizures	8% (small cell lung)
GFAP [59]	68%	50-60	Meningo-encephalo-myelitis or limited forms; optic disc edema; tremor; viral-like prodrome	35% (teratoma)
ITPR1[60]	70%	60-70	Cerebellar ataxia; peripheral neuropathy; encephalitis with seizures; myelopathy	30-40% (breast cancer)
Kelch11[61]	0%	40-50	Ataxia; brainstem dysfunction; encephalopathy	100% (testicular seminoma)
Ma2 (Ta)[62]	32%	60-70	LE; diencephalic syndrome (narcolepsy/cataplexy); brainstem symptoms	90% (testicular tumors)
NfL[63]	50%	60-70	Ataxia; encephalopathy; myelitis	76% (neuroendocrine [small cell lung, Merkel cell])
PCA-2/ MAP1B[64]	70%	60-70	Ataxia; brainstem symptoms; peripheral neuropathy	90% (small cell lung)

Abbreviations: AK5 = adenylate kinase 5; AGNA = anti-glial nuclear antigen; ANNA-1/2 = anti-neuronal nuclear antibodies type-1/2; CRMP5 = collapsin response-mediator protein-5; GAD-65 = glutamic acid decarboxylase-65; GFAP = glial fibrillary acidic protein; GI = gastrointestinal; LE = limbic encephalitis; NfL = neurofilament light chain; PCA-2/ MAP1B = Purkinje cells antigens-2/microtubule-associated protein 1B; SPS = stiff-person syndrome. [Modified from Table 2 in: Sechi E, Flanagan EP. Curr Treat Options Neurol. 2019 Feb 27;21(3):11].

2) EPIDEMIOLOGY OF AQUAPORIN-4-IgG-POSITIVE NMOSD IN SARDINIA

Background

NMOSD is a rare antibody-mediated demyelinating disease of the CNS, distinct from MS.[11] The disease is clinically characterized by recurrent attacks of myelitis (typically longitudinally extensive over >3 contiguous vertebral body segments), optic neuritis (typically recurrent and/or bilateral) and, less commonly, brain/brainstem dysfunction (*e.g.*, area postrema syndrome).[8] Similar clinical-MRI manifestations can be seen in patients with antibodies against the myelin oligodendrocyte glycoprotein (MOG-IgG),[65] or rare patients without detectable antibodies (seronegative NMOSD) of yet unclear etiology.[66, 67] From an epidemiological standpoint, NMOSD has a strong predilection for the female sex and people of Asian or African ancestry, while Countries with a predominantly White population seem less affected.[68, 69] Other proposed risk factors for the disease include infections, dietary habits, smoking, levels of physical activity, low serum vitamin D, and the presence of certain HLA-DRB1 alleles and single nucleotide polymorphisms.[70-73]

The Italian island of Sardinia has extensively been studied for its unique epidemiological characteristics and increased prevalence of certain immune-mediated disorders.[19, 22] In particular, Sardinia is well recognized as a high-risk area for MS, with among the highest incidence and prevalence reported worldwide.[20-23, 74] However, the epidemiology of other less common demyelinating CNS disorders, such as NMOSD, remains unclear. The aim of this study was to determine the incidence and prevalence of NMOSD in Sardinia over a ten-year study period (2013-2022).

Methods

In this retrospective observational study, incidence of NMOSD was calculated between January 1, 2013 and December 31, 2022; while the prevalence day was December 31, 2022. The study was conducted in accordance with the STROND guidelines for the reporting of incidence and prevalence studies in neuroepidemiology.[75] All involved patients consented to the use of their medical records for research.

Study population

Sardinia is an Italian island in the Mediterranean Sea with a surface of 24,100 Km^2. The population on December 31, 2022 was of 1.578.146 residents; 50.9% of whom were female, and >95% White. Patients with MS and other demyelinating CNS disorders are treated and followed by four specialized neurology units across the Sardinian territory. Autoantibody testing for AQP4-IgG is generally performed at the Laboratory of the MS Center in Cagliari (reference and only laboratory for AQP4-IgG testing in Sardinia) by fixed cell-based assay (CBA), although serum samples may occasionally be sent to other laboratories outside the island.

Identification of cases

A flow-chart summarizing the process of identification and inclusion of cases is shown in **Figure 1**. Patients were identified through two main sources. We first searched the archives of the Laboratory of the MS Center in Cagliari to identify patients with AQP4-IgG positivity among 2017 unique patients tested between December 2009 (first availability of AQP4-IgG testing in Sardinia) and December 2022. Additional patients with a diagnosis of NMOSD and antibody positivity determined in laboratories outside the island were identified by reviewing medical records of the four hospitals with neurology units specialized in the treatment of MS and other CNS demyelinating

disorders: 1) MS Center - Binaghi Hospital of Cagliari; 2) Neurology Unit - Brotzu Hospital of Cagliari; 3) Neurology Unit - San Francesco Hospital of Nuoro; and 4) Adult and Pediatric Neurology Units - University-Hospital of Sassari (the Child Neurology Unit of the University Hospital of Sassari is the reference Sardinian unit for treatment of pediatric MS). For each identified patient, the demographics, residency status and clinical information were abstracted from medical records, and the clinical-MRI characteristics reviewed to confirm the diagnosis of NMOSD based on the 2015 diagnostic criteria by the International Panel for NMO Diagnosis.[8] Cases meeting the criteria for seronegative NMOSD, with or without known positivity for MOG-IgG, were not included in the study. Duplicates were identified by using the Italian tax codes.

Inclusion and exclusion criteria

Identified cases with a diagnosis of NMOSD were included in the study if they met the following inclusion criteria: 1) Disease onset between January 1, 2013 – December 31, 2022 while resident in Sardinia (incident case); and/or 2) residency in Sardinia on December 31, 2022 (prevalent case). Patients with disease onset before 2013 who moved outside Sardinia or died by December 31, 2022 were excluded. Two patients were previously reported in form of case report.[76, 77]

Antibody testing

Serum positivity for AQP4-IgG was assessed by commercial (Euroimmun®) fixed CBA in all patients except one, for which positivity was initially detected by tissue immunohistochemistry and later confirmed by live CBA (Dalmau's Laboratory, Barcellona).[76]

Statistical analysis

Prevalence was calculated on December 31, 2022, as the number of NMOSD cases per 100,000. Incidence rate was defined as the ratio of new patients with NMOSD to the

number of person-years at risk during the study period (2013-2022), reported per 1 million person-years. Epidemiological measures were reported with 95% Confidence Intervals (CI). Incidence Rate Ratio (IRR) was calculated to compare incidence rates of events between two study periods (2013-2017 VS. 2018-2022). Differences in prevalence and incidence rates were assessed using Fisher exact and Chi-squared tests; Poisson distribution and test-based methods were used to construct the confidence intervals.

Age standardization to the European and World populations was performed. Data from Census performed by Statistic National Italian Institute (ISTAT) were used for age and sex strata calculation for the Sardinian population. Statistical significance was set at $p<0.05$; OpenEpi version 3.0, MedCALC and STATA17 software's were used for statistical computation.

Results

A total of 45 cases were included in the study (incident, 31; prevalent, 41); their demographics and clinical characteristics are summarized in **Table 3**.

Incidence and prevalence estimates

The crude incidence (95% CI) of NMOSD in Sardinia over ten years (2013-2022) was 1.9 (1.3-2.7) per million person-years, while the prevalence on December 31, 2022 was 2.6 (1.9-3.5) per 100,000. The disease prevalence increased significantly from January 1, 2013 (1.1 per 100,000 = 18 cases over 1,640,379 inhabitants) to December 31, 2022 (2.6 per 100,000 = 41 cases over 1,578,146 inhabitants), $p=0.002$. The IRR was 0.88 (95% CI: 0.40-1.90); comparison of incidence rates between 2013-2017 (2.0; 95% CI: 1.1-3.4) and 2018-2022 (2.2; 95% CI: 1.3-3.6) did not reveal statistically significant differences, $p=0.73$.

The detailed distribution of identified cases over the population at risk with relative crude incidence and prevalence estimates, stratified by age and sex, is shown in **Table 4**. The occurrence of new (incident) cases during the study period is schematically represented in **Figure 2**; while **Figure 3** shows the variation in annual incidence and prevalence during the study period.

The age-standardized incidence and prevalence (95% CI) to the European population were 1.7 (1.1-2.4) per million and 2.3 (1.6-3.0) per 100,000; while the age-standardized incidence and prevalence to the World population were 1.3 (0.7-2) per million person-years and 1.8 (1.3-2.3) per 100,000, respectively. **Table 5** compares the crude and age-standardized estimates, and the demographic distribution of the Sardinian, European and World populations.

Clinical characteristics of incident cases

Among incident cases with detailed clinical information available, the median age at disease onset was 54 (range, 17-78) years, with only 1 (3%) patient presenting before the age of 18 years; 29 (96%) were female (**Table 3**).

The presenting clinical attack were longitudinally extensive transverse myelitis in 21 (68%), optic neuritis in 5 (16%; bilateral in one case), brainstem dysfunction in 2 (6%; area postrema syndrome in one case), encephalitis in one (3%), or combinations thereof in 2 (6%; one case had myelitis with area postrema syndrome, while the other had unilateral optic neuritis with area postrema syndrome). Concomitant autoimmune disorders were reported in 16 (52%) cases, including autoimmune thyroiditis (n=10), Sjögren's syndrome (n=3), celiac disease (n=3), rheumatoid arthritis (n=2), psoriasis (n=1), systemic lupus erythematosus (n=1), vitiligo (n=1), type I diabetes (n=1), autoimmune thrombocytopenia (n=1), and myasthenia gravis (n=1). One patient had a concomitant positivity for myelin oligodendrocyte (MOG)-IgG in serum, assessed by

both fixed (Euroimmun®) and live CBA (serum titer, 1:160; CSF titer, 1:2; test performed at the Neuropathology Laboratory of Verona, Italy).

This patient with dual positivity for AQP4-IgG and MOG-IgG was a 72-year-old woman who developed a severe, longitudinally extensive transverse myelitis requiring wheelchair at nadir. On MRI, the spinal cord T2-lesion extended from C2 to T9 and showed intralesional spots of higher T2-hyperintensity, typically seen with AQP4-IgG. Irregular enhancement of the lesion was observed after gadolinium. The patient was treated acutely with intravenous methylprednisolone (1 g/day for 5 days) and plasma exchange (6 total exchanges, every other day) with good clinical recovery; and long-term with rituximab without further relapses after 3 years. The spinal cord T2-abnormalities did not resolve at MRI follow-up. Ten additional patients were tested for MOG-IgG as part of their diagnostic workup, with negative result.

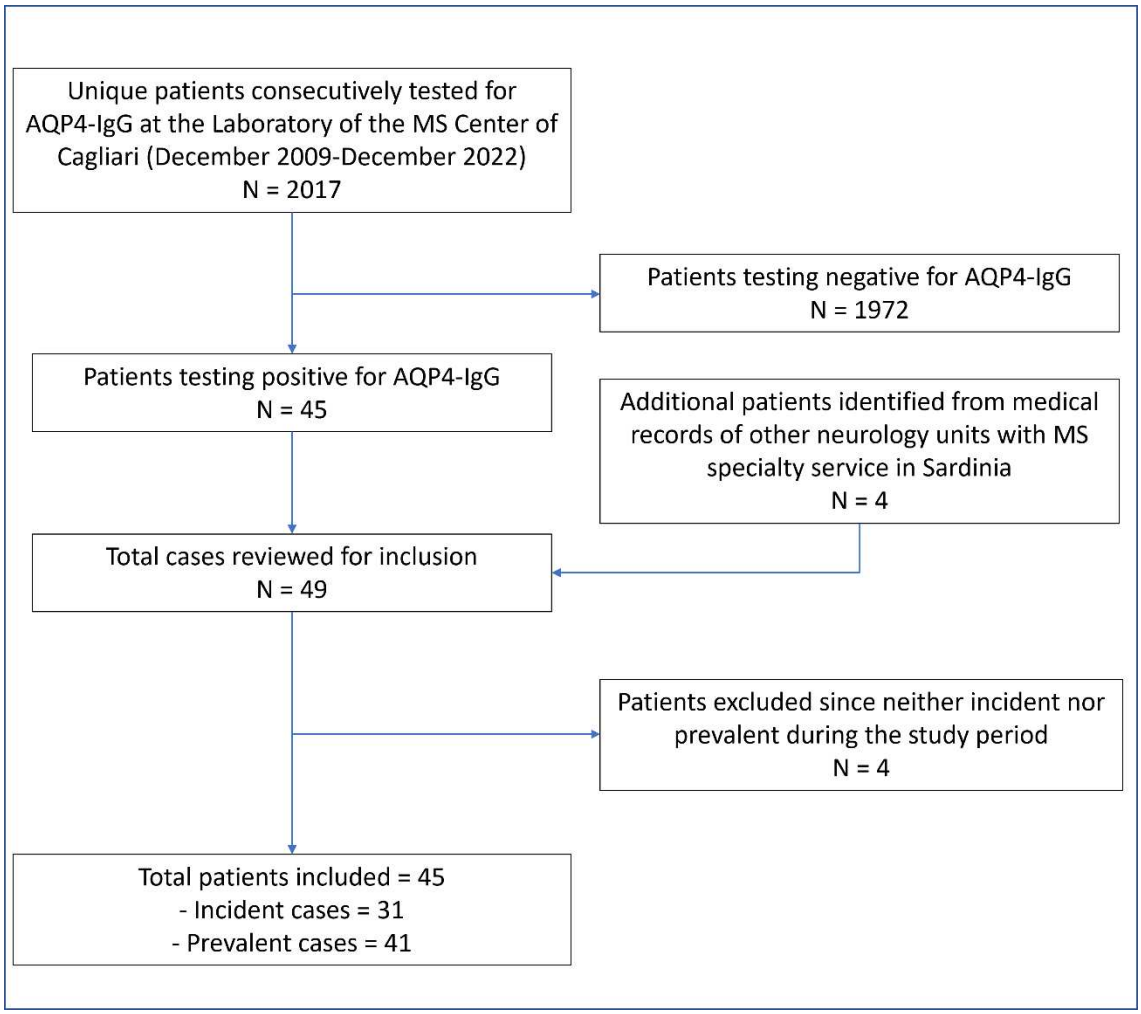

Figure 1 – Flow-chart for identification and inclusion of patients with NMOSD

Most patients were initially identified by searching the archives of the Laboratory of the MS Center in Cagliari (reference and only laboratory for AQP4-IgG testing in the entire island since December 2009). Four additional patients with a diagnosis of NMOSD and positivity detected in other laboratories outside Sardinia were identified by searching medical records of the four reference hospitals with dedicated MS units in the island (including the reference Sardinian unit for pediatric MS at the University Hospital of Sassari). Out of 49 patients initially identified, four were excluded as neither incident nor prevalent for the 2013-2022 study period. A total of 45 cases were eventually included (incident, 31; prevalent, 41).

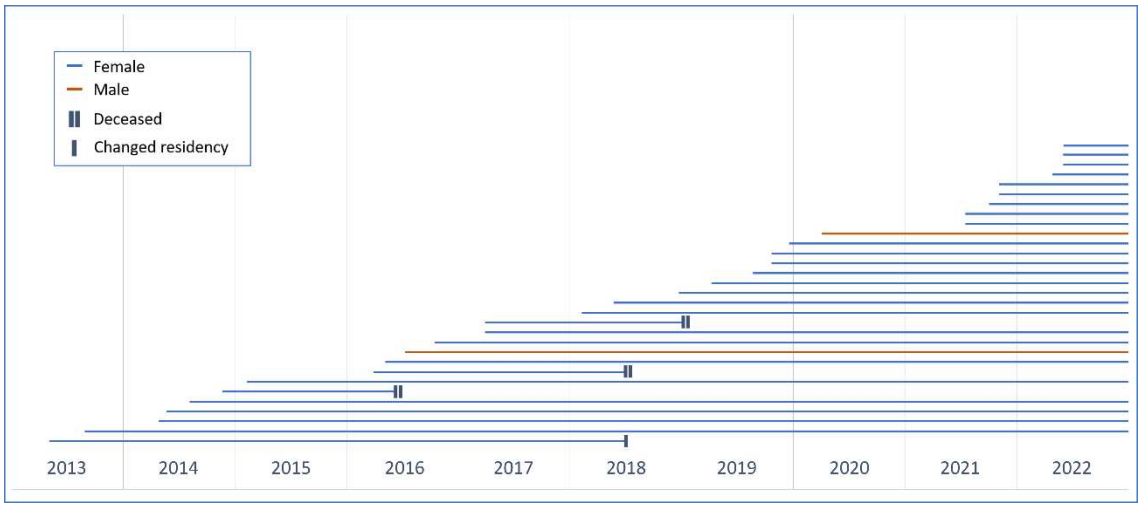

Figure 2 – Temporal distribution of incident NMOSD cases over time

The horizontal lines in the graph represent incident cases occurring over the study period (2013-2022). Female patients are represented by blue lines, while brown lines represent males. Certain lines are interrupted due to death of the patient (double vertical bars) or change of residency outside Sardinia (single vertical bar).

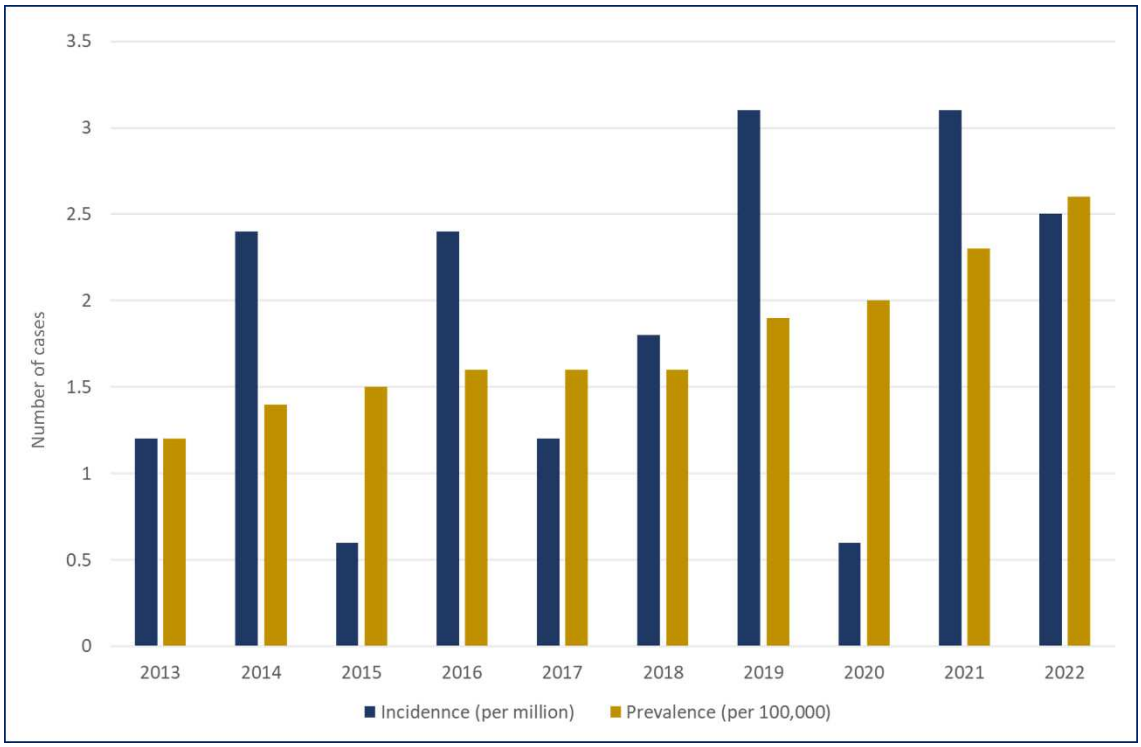

Figure 3 – Variation in annual incidence and prevalence of NMOSD over ten years

The bar graph shows the variations in annual incidence (blue bars) and prevalence (golden bars) of NMOSD in Sardinia between 2013 and 2022. Note that while incidence varies substantially over the study period, the annual prevalence steadily increases from 2013 to 2022.

Table 3 – Demographics and clinical characteristics for the full cohort of included cases with NMOSD and for incident cases only; quantitative and qualitative variables are reported as median (range) and frequencies (%)

	Full cohort (N=45)	Incident cases (N=31)
Age at disease onset	51 (6-78)	54 (17-78)
Onset age <18 years	3 (7%)	1 (3%)
Onset age 18-39 years	9 (20%)	4 (13%)
Onset age 40-64 years	21 (46%)	14 (45%)
Onset age 65+ years	12 (27%)	12 (49%)
Female sex	40 (89%)	29 (96%)
Ethnicity		
White	43 (96%)	30 (97%)
Black	1 (2%)	0 (0%)
Asian	1 (2%)	1 (3%)
Coexisting autoimmunity	23 (51%)	16 (52%)
Number of relapses	2 (1-15)	2 (1-10)
Presenting attack		
LETM	26 (57%)	21 (68%)
ON	13 (29%)	5 (16%)
Brain/brainstem attack	3 (7%)	3 (10%)
Combinations of the above	3 (7%)	2 (6%)
CSF-restricted OCB (≥2)	4/27 (15%)	3/20 (15%)
EDSS at last follow-up	4 (0-8.5)	3 (0-8.5)
Follow-up duration, years	6 (1-27)	3 (1-9)

Abbreviations – CSF = cerebrospinal fluid; EDSS n= expanded disability status scale-score; LETM = longitudinally extensive transverse myelitis; OCB = oligoclonal bands; ON = optic neuritis.

Table 4 – Incidence and prevalence estimates for Sardinia stratified by age and sex

Incident NMOSD cases (person-years at risk) for 2013–2023			
Age strata	Female	Male	tot
< 18	1 (1,080,705)	0 (1,163,215)	1 (2,243,920)
18 – 39	3 (1,908,395)	1 (2,009,330)	4 (3,917,725)
40 – 64	13 (3,140,870)	1 (3,018,875)	14 (6,227,150)
≥ 65	12 (2,112,805)	0 (1,729,450)	12 (3,774,850)
tot	28 (8,242,775)	2 (7,920,870)	31 (16,163,645)
NMOSD incidence in Sardinia (95% CI) per 1 million person-years for 2013–2023			
Age strata	Female	Male	tot
< 18	0.9 (0.5-4.6)	0 (0.0-0.0)	0.5 (0.0-2.2)
18 – 39	1.6 (0.4-4.3)	0.5 (0.0-2.5)	1.0 (0.3-2.5)
40 – 64	4.1 (2.3-6.9)	0.3 (0.0-0.6)	2.3 (1.3-3.7)
≥ 65	5.7 (3.1-9.7)	0 (0.0-0.0)	3.2 (1.7-5.4)
tot	3.4 (2.3-4.8)	0.3 (0.0-0.8)	1.9 (1.3-2.7)
Prevalent NMOSD cases (population) on December 31, 2022			
Age strata	Female	Male	tot
< 18	0 (98,713)	0 (105,849)	0 (204,562)
18 – 39	4 (162,888)	3 (174,048)	7 (336,936)
40 – 64	18 (312,523)	2 (310,002)	20 (622,525)
≥ 65	14 (229,777)	0 (184,346)	14 (414,123)
Total	36 (803,901)	5 (774,245)	41 (1,578,146)
NMOSD prevalence in Sardinia (95% CI) per 100,000 on December 31, 2022			
Age strata	Female	Male	tot
< 18	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)
18 – 39	2.5 (0.8-5.9)	1.7 (0.4-4.7)	2.1 (0.91-4.11)
40 – 64	5.8 (3.5-8.9)	0.7 (0.1-2.1)	3.2 (2.0-4.9)
≥ 65	6.1 (3.5-10.0)	0 (0.0-0.0)	3.4 (1.9-5.5)
Total	4.5 (3.2-6.1)	0.7 (0.2-1.4)	2.6 (1.9-3.5)

Abbreviations: CI = confidence interval; NMOSD = aquaporin-4-IgG positive neuromyelitis optica spectrum disorder; WHO = World Health Organization The table shows the characteristics of the Sardinian population during the study period (2013–2022) and at prevalence day (December 31, 2022), stratified by age and sex with relative incidence and prevalence estimates for NMOSD. Incidence was calculated assuming that incident cases occurred as observations of a Poisson random variable. The ISTAT Sardinia population estimates were used for age and sex strata calculation (website). Person-years are computed by averaging the Sardinian population on January 1, 2013 and on December 31, 2022, and multiply by 10.

Table 5 – Incidence and prevalence NMOSD estimates for Sardinia and after age-standardization to the European and World population, and comparison of the three populations

Incidence/million and prevalence/100,000 (95% CI) estimates for Sardinia and after age-standardization to the World and European population			
	Sardinia	Age-standardized values to the European Region	Age-standardized values to the World
Incidence	1.9 (1.3-2.7)/million	1.8 (1.1-2.4)/million	1.3 (0.7-2.0)/million
Prevalence	2.6 (1.9-3.5)/100,000	2.3 (1.6-3.0)/100,000	1.8 (1.3-2.3)/100,000
Age strata in the Sardinian, European and World populations			
Age strata	Sardinia (Dec 31, 2022), n (%)	Europe standard population (ESP 2011-2030), n (%)*	World standard population (WHO 2000–2025), n (%)*
< 18	204,562 (13)	215,000 (21) §	312,448 (31)
18 – 39	336,936 (21)	255,000 (25) §	342,622 (35)
40 - 64	622,525 (39)	335,000 (35)	262,608 (26)
≥ 65	414,123 (26)	195,000 (19)	82,322 (8)
Total	1,578,146	1,000,000	1,000,000

Abbreviations: CI = confidence interval; NMOSD = aquaporin-4-IgG positive neuromyelitis optica spectrum disorder; WHO = World Health Organization
*Data sources for European and World standard population available at: https://www.opendata.nhs.scot/es/dataset/standard-populations; and seer.cancer.gov/stdpopulations/world.who.html.
§ For European data the two classes were defined as follow: < 19- and 20-39 years.

3) EPIDEMIOLOGY OF SEROPOSITIVE MYASTHENIA GRAVIS IN SARDINIA: A POPULATION-BASED STUDY IN THE DISTRICT OF SASSARI

Background

MG is an autoimmune disorder characterized by abnormal muscle fatigability due to antibody-mediated dysfunction of the neuromuscular junction. AChR-IgG and MuSK-IgG are detected in approximately 80% and 5% of patients, respectively (referred to hereafter as "seropositive" MG). Epidemiological studies on MG variably showed an incidence of 6-31/million, and a prevalence of 10-37/100,000.[14, 78]

The Italian island of Sardinia is recognized for the high risk of different immune-mediated disorders (*e.g.*, multiple sclerosis, type-I diabetes),[19, 23] but epidemiological data on MG are scarce. We determined the epidemiology of seropositive MG in the district of Sassari (Northwestern Sardinia).

Methods

The study was approved by the Institutional Review Board of the University of Cagliari. All involved patients consented to use of their medical records for research.

Study population and setting

Sardinia has a predominantly White population (>95%), with 1,611,621 inhabitants; 50.9% female.[79] A total of eight public neurology hospital units serve the region, including one reference unit for child neurology. Autoantibody testing for AChR-IgG and MuSK-IgG by radio-immune assay (RIA) first became available in 1998 and 2005, respectively, at the laboratory of the University-Hospital of Sassari. After 2014, AChR-IgG testing by RIA also became available at the Brotzu Hospital in Cagliari (South Sardinia), and other minor laboratories.

The sanitary district of Sassari (325,288 inhabitants) includes the University-Hospital of Sassari and its laboratory. This study was conducted in the district of Sassari as representative of the Sardinian population.

Identification of patients

The steps toward identification and inclusion of patients are summarized (**Figure 4**). Patients testing positive for AChR-IgG and/or MuSK-IgG at the laboratory of the University-Hospital of Sassari between April 1998-February 2022 represented the main study source. Additional data were integrated from the following sources: 1) Records of AChR-IgG-positive patients of the Brotzu Hospital since 2014; and 2) medical records of 6/7 adult neurology units, and of the reference child neurology unit in Sardinia. Patients known to be diagnosed/followed at reference MG centers outside Sardinia were also considered. Duplicates were identified by using the Italian tax identification codes.

Inclusion and exclusion criteria

Of identified patients with AChR-IgG/ MuSK-IgG positivity, medical records were reviewed (ES, PZ, PC) to include those meeting the following criteria: 1) Clinical symptoms/signs consistent with MG; 2) onset between January 1, 2010 and December 31, 2019, while resident in the district of Sassari (incident cases); and/or 3) residence in the district of Sassari on December 31, 2019 (prevalent cases). Residents with onset before 2010 who moved outside the district or died by December 31, 2019 were excluded. We also excluded patients with clinical manifestations not consistent with MG, or for whom insufficient information was available.

Autoantibody testing

Seropositivity for AChR-IgG and MuSK-IgG was assessed by RIA with positivity titer-thresholds of ≥0.5 nmol/L and ≥100 pmol/L, respectively. The first available positive test per patient was used for descriptive statistics.

Statistical analysis

Continuous and categorical variables were summarized as median (range) or number (percentage), and compared using the U-Mann Whitney or Chi-square/Fisher's exact tests, as appropriate.

Prevalence was calculated on December 31, 2019, as number of cases per 100,000. Incidence was described as the number of new cases per million person-years at risk, assuming they occurred following a Poisson distribution; 95% confidence intervals (CI) were calculated. Person-years at risk were computed by averaging the Sassari district population for the years 2010 and 2019 and multiplying by ten.[79] Age-standardization to the world population was performed, according to the WHO.[80] Data were analysed using OpenEpi 3.0 and; STATA17 (StataCorp LLC., College Station, TX, USA).

Results

A total of 202 patients were included: 180 prevalent cases and 107 incident cases.

Table 6 summarizes their clinical characteristics. One patient showed double positivity for MuSK-IgG (titer, 1600 pmol/L) and AChR-IgG (titer, 0.6 nmol/L).

Incidence and prevalence of seropositive MG

Table 7 shows the crude MG incidence and prevalence estimates stratified by age and sex. Antibody-specific estimates are summarized in **Table 8** and **Table 9**. Disease prevalence in the district of Sassari significantly increased from 2010 (28.6/100,00) to 2019 (55.3/100,000), p=<0.0001. After age-standardization to the world population, the crude incidence and prevalence estimates of seropositive MG decreased substantially due to wider representation of older age strata (≥50 years) in the Sardinian population (48%) compared to the world population (22%) (**Table 10**).

Mobility between Sardinian districts

The municipality of residence at MG presentation remained the same until the prevalence day (or death) in 192/202 cases, suggesting low risk of bias due to mobility between Sardinian districts. Of the remaining 10 patients, the municipality of residence at onset was unknown for 5 prevalent cases, 4 patients moved to a different municipality in the district of Sassari, and one patient moved into the district after MG presentation.

Characteristics of incident cases

Detailed clinical characteristics were available for most of the 107 incident cases. The majority presented at age 50 years or older, and were male; MG onset before 18 years of age was observed in only 2% of patients (**Figure 5**).

Fifty-one patients (48%) presented as ocular MG: 41 had available follow-up details, 15 (37%) of whom developed extraocular symptoms during the study period, after a median of 13 (range, 1-34) months. Among patients with AChR-IgG, the median antibody titer (nmol/L) was significantly higher in those presenting as generalized MG (7.8; IQR, 5.1-11.3) vs those presenting as ocular MG (5.5; IQR, 3.3-7.5); p=0.003.

Data on repetitive nerve stimulation or single fiber EMG, variably performed at different sites, were available for 75/107, showing abnormalities in 34: generalized MG, 25/46 (54%); ocular MG, 9/29 (31%). Coexisting autoimmune/immune-mediated disorders were reported in 20 patients (Table 1), including thyroiditis (n=12), encephalitis (n=2; with N-methyl-D-aspartate receptor and glutamic acid decarboxylase-65 autoantibodies, respectively), cholangitis (n=2), psoriatic arthritis (n=2), thrombocytopenia (n=1), systemic lupus erythematosus (n=1), ulcerative colitis (n=1), vitiligo (n=1), and type-I diabetes (n=1).

Chest CT, available for 101/107 cases, showed abnormalities in 28. Ten patients eventually received a diagnosis of thymoma (pathologically confirmed, n=9; presumed due to metastatic disease, n=1).

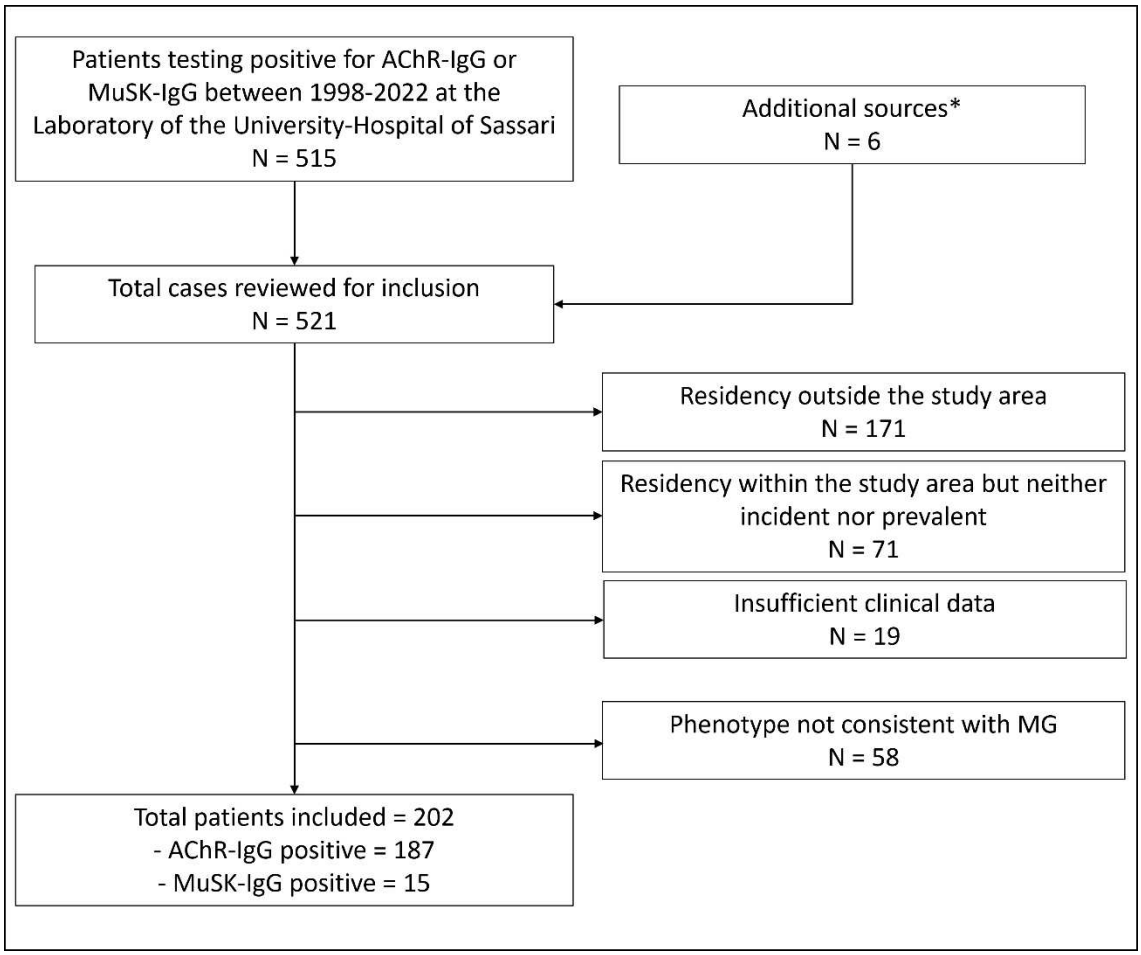

Figure 4 – Steps towards identification of seropositive MG patients

The flow-chart summarizes the steps towards identification of seropositive MG patients from different sources to their inclusion in the study. The archives of the laboratory of the University-Hospital of Sassari represented the main study source. *Of the six patients identified through additional sources, 2 were identified from medical records of neurology hospital units in the north of Sardinia, while 4 were known to be followed at reference MG centers outside Sardinia.

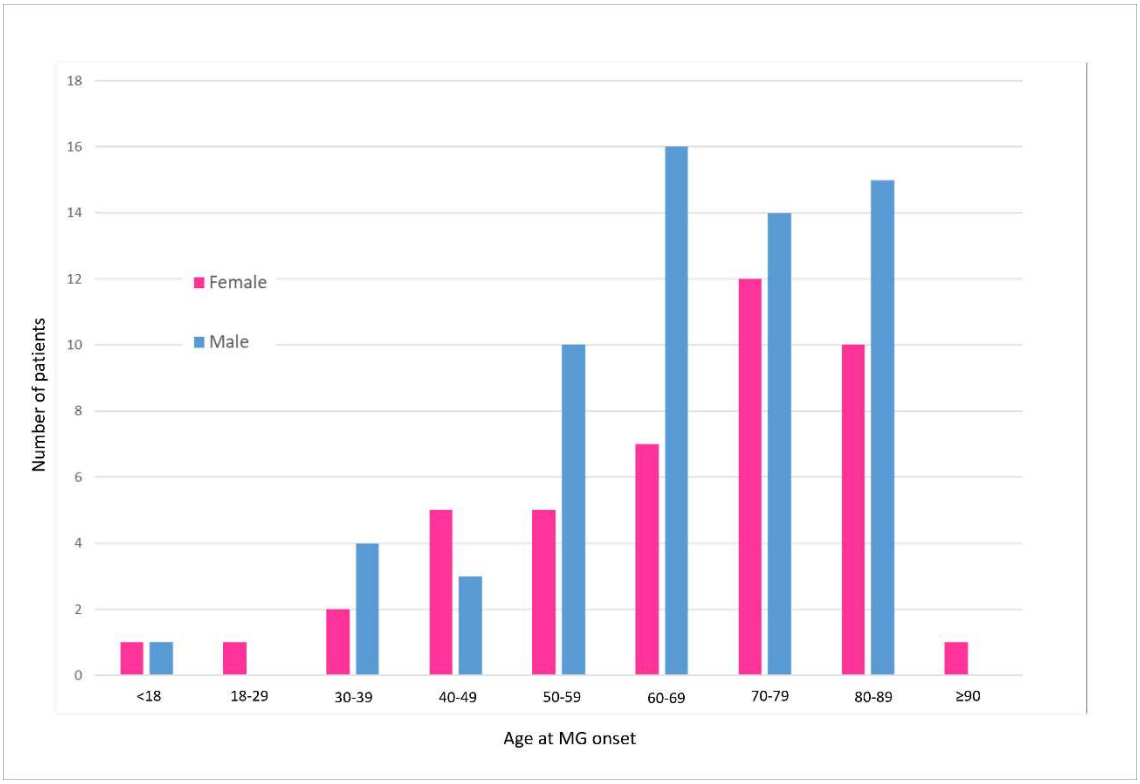

Figure 5 – Distribution of incident cases by age and sex at MG presentation

The bar graph shows the distribution of incident cases at onset of seropositive MG in Sardinia over 10 years (2010-2019), stratified by sex. An increase in the incidence of male patients is observed after 50 years of age, while a slight female predominance seems to occur at younger ages.

Table 6 – Demographic and clinical characteristics of included cases, stratified by incident cases and full cohort (including incident cases)

Variables		Full cohort (n=202)	Incident cases (n= 107)
Male sex, n (%)		100 (50)	63 (59)
Onset age, years (range)		57 (7-90)	70 (8-90)
MG age classes, n (%)	< 18	10 (5)	2 (2)
	18 - 49	67 (33)	15 (14)
	50 - 64	45 (22)	23 (22)
	≥ 65	80 (40)	67 (63)
Onset phenotype, n (%)	GMG	108 (55) *	56 (52)
	OMG	90 (45) *	51 (48)
Phenotype – December 2019, n (%)	GMG	131 (76) *	60 (72) *
	OMG	42 (24) *	23 (28) *
Autoantibody, n (%)	AChR-IgG	187 (93)	102 (95)
	MuSK-IgG	15 (7)	5 (5)
Coexisting autoimmunity, n (%)		-	20/78 (26) *

Abbreviations: AChR = acetylcholine receptor; GMG = generalized myasthenia gravis; MuSK = muscle specific kinase; OMG = ocular myasthenia gravis;
* Sufficient clinical information was not available for all patients

Table 7 – Incidence and prevalence MG estimates for the district of Sassari, stratified by age and sex

MG incidence (95% CI) per 1 million person-years for 2010–2019			
Age at onset (years)	Female	Male	Total
< 18	4.4 (0.2-21.5)	4.1 (0.2-20.3)	4.2 (0.7-14)
18 – 49	12 (5.6-22.7)	10.2 (4.4-20.1)	11.1 (6.4-17.8)
50 – 64	16.2 (6.6-33.7)	47.8 (28.9-74.9)	31.7 (20.6-46.7)
≥ 65	70.5 (48-99.9)	120.3 (86.4-163.5)	92.1 (72.0-116.3)
Total	26.2 (19.3-34.8)	39.3 (30.4-49.9)	32.6 (26.8-39.2)
MG prevalence (95% CI) per 100,000 on December 31, 2019			
Age (years)	Female	Male	Total
< 18	4.7 (0.2-22.9)	0.0 (0.0-0.0)	2.3 (0.1-11.1)
18 – 49	31.4 (19.4-48.1)	21.9 (12.4-35.8)	26.5 (18.5-36.8)
50 - 64	68.5 (46.1-98.3)	47.7 (29.1-73.9)	58.3 (43.0-77.3)
≥ 65	92.3 (67.1-12.4)	172.5 (132.8-220.5)	127.5 (104.4-154.3)
Total	53.0 (42.5-65.1)	57.7 (46.8-70.5)	55.3 (47.7-63.9)

Abbreviations: CI = confidence interval; MG = myasthenia gravis.

Table 8 – Incidence and prevalence estimates for AChR-IgG-positive MG in the District of Sassari, stratified by age and sex

AChR-IgG MG incidence (95% CI) per 1 million person-years for 2010–2019			
Age at onset (years)	Female	Male	Total
< 18	4.4 (0.2-21.5)	4.1 (0.2-20.3)	4.2 (0.7-14.0)
18 – 49	10.5 (4.9-20.7)	7.3 (2.7-16.1)	8.8 (4.8-15.0)
50 - 64	16.2 (6.6-33.7)	45.0 (26.6-71.4)	30.3 (19.5-45.1)
≥ 65	68.0 (46.1-97.0)	120.3 (86.4-163.5)	90.7 (70.7-114.7)
Total	25.0 (18.2-33.5)	37.4 (28.8-47.8)	31.1 (25.4-37.5)
AChR-IgG MG prevalence (95% CI) per 100,000 on December 31, 2019			
Age (years)	Female	Male	Total
< 18	4.7 (0.2-22.9)	0 (0.0-0.0)	2.3 (0.1-11.1)
18 – 49	26.4 (15.6-42.0)	15.6 (7.9-27.8)	20.9 (13.9-30.1)
50 - 64	60.9 (39.9-89.2)	47.7 (29.1-73.9)	54.4 (39.7-72.9)
≥ 65	85.6 (61.4-116.2)	169.6 (130.3-217.3)	122.5 (99.9-148.8)
Total	47.6 (38.0-59.0)	54.6 (43.4-67.0)	51.0 (43.7-59.3)

Abbreviations: CI = confidence interval; MG = myasthenia gravis.

Table 9 – Incidence and prevalence estimates for MuSK-IgG-positive MG in the District of Sassari, stratified by age and sex

MuSK-IgG MG incidence (95% CI) per 1 million person-years for 2010–2019			
Age at onset (years)	Female	Male	Total
< 18	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)
18 – 49	1.5 (0.1-7.4)	2.9 (0.5-9.6)	2.2 (0.6-6.0)
50 - 64	0 (0.0-0.0)	2.8 (0.1-13.9)	1.4 (0.1-6.8)
≥ 65	2.4 (0.1-12.0)	0 (0.0-0.0)	1.4 (0.1-6.8)
Total	1.2 (0.2-3.9)	1.9 (0.5-5.1)	1.5 (0.6-3.4)
MuSK-IgG MG prevalence (95% CI) per 100,000 on December 31, 2019			
Age (years)	Female	Male	Total
< 18	0 (0.0-0.0)	0 (0.0-0.0)	0 (0.0-0.0)
18 – 49	5.0 (1.3-13.5)	6.3 (2.0-15.1)	5.6 (2.5-11.1)
50 - 64	7.6 (1.9-20.7)	0 (0.0-0.0)	3.9 (1.0-10.6)
≥ 65	6.8 (1.7-18.4)	2.9 (0.1-14.2)	5.1 (1.6-12.2)
Total	5.4 (2.7-10.0)	3.1 (1.2-7.0)	4.3 (2.5-7.1)

Abbreviations: CI = confidence interval; MG = myasthenia gravis.

Table 10 – Comparison of incidence and prevalence MG estimates for the District of Sassari and after standardization to the World population

Incidence/million and prevalence/100,000 (95% CI) estimates for the District of Sassari and after age-standardization to the World population		
	District of Sassari (Sardinia)	World
Overall incidence	32.6 (26.8-39.2)	18.4 (14.3-22.5)
Overall prevalence	55.3 (47.7-63.9)	31.6 (26.1-37.0)
AChR MG incidence	31.1 (25.4-37.5)	17.1 (13.2-20.9)
AChR MG prevalence	51.0 (43.7-59.3)	28.0 (23.0-33.0)
MuSK MG incidence	1.5 (0.6-3.4)	1.3 (0.1-2.6)
MuSK MG prevalence	4.3 (2.5-7.1)	3.6 (1.5-5.7)
Age strata distribution in the Sardinian and World populations		
Age classes (years)	Sardinia (Dec 31, 2019), n (%)	World standardized million (WHO 2000–2025), n (%)
< 18	217,811 (14)	312,448 (31)
18 – 49	616,961 (38)	468,878 (47)
50 - 64	383,036 (24)	136,352 (14)
≥ 65	393,813 (24)	82,322 (8)
Total	1,611,621	1,000,000

Abbreviations: CI = confidence interval; MG = myasthenia gravis.

4) DISCUSSION

In this project, we have determined the incidence and prevalence of two of the most common antibody-mediated neurologic disorders in the Italian region of Sardinia. Our findings provide important insights for better understanding of the determinants of the high risk of certain autoimmune and immune-mediated neurologic disorders in Sardinia, and might guide planning future allocation of healthcare resources. Data from this project resulted in the publication of two peer-reviewed articles.[81, 82]

The **first aim** of this project was to study the epidemiology of NMOSD in Sardinia. We found the incidence and prevalence of NMOSD in Sardinia are overall in line with those of other Countries with a predominantly White population. This is in contrast with the exceedingly high risk of MS historically documented in the island,[20, 21, 23] and may suggest different genetic and/or environmental risk factors for these two demyelinating CNS disorders.[71, 73, 83, 84]

The incidence and prevalence of NMOSD worldwide range between 0.5-6.5 per million person-years and 0.5-7.9 per 100,000, respectively, with the highest values observed among East-Asians and African-Caribbeans.[85-89] Studies on predominantly White populations, including other European Countries, consistently reported a disease incidence around 1/million person-years, which is overall in line with what observed in our study. The reported disease prevalence among Whites is also comparable to our findings, ranging from 0.8-1.6 per 100,000 in Europe to 3.3 per 100,000 in Olmsted County (USA).[85, 90-92] Notably, in the Olmsted County study 84% of cases with demyelinating CNS disorders in the study population were tested for AQP4-IgG, which

might have allowed identification of patients with longstanding disease who potentially were not recognized before the test was available.[85]

The global incidence and prevalence of NMOSD vary widely, and an inverse relationship between the frequency of NMOSD and MS seems to exist. In Countries with high incidence and prevalence of NMOSD, the relative frequency of MS among demyelinating CNS disorders is usually lower with a NMOSD to MS ratio of up to 4:1;[93] while the ratio decreases to approximately 1:50 among Whites.[85] The high MS prevalence reported in Sardinia (330 per 100,000 on March 31, 2016) is in line with these findings,[23] and would correspond to a NMOSD to MS ratio of 1:79, accepting temporal differences. Taken together, these data suggest an increased disease-specific risk for MS rather than a generic predisposition for immune-mediated CNS disorders in the Sardinian population. Notably, some of the proposed environmental risk factors for NMOSD are similar to those proposed for MS (*e.g.*, infections, smoking, vitamin D deficiency), suggesting the difference in frequency between the two diseases in Sardinia may mostly reflect differences in the genetic background.

The characteristics of Sardinian patients with NMOSD are overall in line with prior studies, including the high female:male ratio of 9:1,[85] and low frequency of pediatric involvement around ≤5% of total patients.[94] However, t some peculiarities in our cohort deserve to be highlighted. The expected median age at disease onset in patients with NMOSD is approximately 40 years, while in this study we found a median of 51 years, with only 27% of patients (17% if only incident cases are considered) presenting before the age of 40. This difference might be explained by the longevity of the Sardinian population with higher representation of older age strata compared to the World population (Table 3).[16] The older age in our cohort might also account for the high frequency of incident cases presenting with isolated myelitis (70%), which is known to

occur more commonly among late-onset cases.[95] Coexisting autoimmune disorders are common in NMOSD, accounting for 20-30% of cases.[96] In our cohort, however, 50% of cases had coexisting autoimmune disorders (mostly autoimmune thyroiditis), which is in line with the known higher prevalence of other immune-mediated systemic diseases in the Sardinian population (*e.g.*, type 1 diabetes, autoimmune thyroiditis).[17, 19, 97] One patient had a dual positivity for AQP4-IgG and MOG-IgG, which is reported to occur in <1% of patients with AQP4-IgG positivity.[98] In these rare patients, the clinical phenotype is generally more compatible with NMOSD rather than MOGAD, as observed in our case (*e.g.*, isolated myelitis lesion extending over the cervical-thoracic region but sparing the conus; presence of "bright spotty lesions" on MRI spine; lack of T2 lesion resolution over time).

Our study on NMOSD is limited by the retrospective design and selective inclusion of patients with an already established diagnosis of NMOSD (*i.e.*, we might have lost prevalent cases with a longstanding disease predating availability of AQP4-IgG testing). However, an extensive screening for AQP4-IgG antibodies on all patients with CNS demyelinating disease in the entire Sardinian population is prohibitive and it would be unlikely to significantly alter the number of incident cases identified since 2013 (diagnostic attention for the disease has increased during the last two decades). Patients' identification was based on local founts and we might have overlooked patients diagnosed outside of Sardinia, although this seems unlikely given the severity of NMOSD attacks that often require prompt admission to local hospitals, where awareness of the disease is generally high. Our findings might not be generalizable to other Italian regions given the different genetic background of the Sardinian population. Different from some prior epidemiology studies, we did not include patients with a diagnosis of seronegative NMOSD as these forms are increasingly recognized to have

different pathophysiology than NMOSD, including MOG-IgG-associated disease (MOGAD) or other yet unidentified etiologies (either immune-mediated or not).[66, 67, 99] Furthermore, commercial testing for MOG-IgG only recently became available in Sardinia and retrospective testing of patients with suspected phenotype would not be reliable as patients may serorevert to MOG-IgG negative after disease attacks, which is rarely seen with AQP4-IgG.[65, 100] Future prospective studies will better investigate the epidemiology of MOGAD and seronegative NMOSD in Sardinia. Lastly, most patients in our study were not tested for AQP4-IgG by live CBA, which have a higher sensitivity compared to the commercial fixed CBA,[101] and a minority of patients might have been unrecognized.

The **second aim** of this project was to indirectly address the epidemiology of seropositive MG in Sardinia, by conducting a population-based study in the Sanitary District of Sassari. We found that Sardinia is a high-risk area for MG, with a prevalence that exceeds the European threshold for definition of rare disease (*i.e.*, 50/100,000). Identification of the determinants of this risk may inform our understanding of disease pathophysiology.

The incidence and prevalence of MG have increased during the last 20 years due to wider availability of autoantibody testing and greater awareness among clinicians.[14] Comparisons of epidemiological studies are often challenging owing to different methodologies (*e.g.*, selective inclusion of adult or seropositive patients) and eras,[102-104] and updated epidemiological studies worldwide are needed. Data on MG epidemiology in Italy and Sardinia are scarce.[105-108] A small study in 2012 using data from 21 general practitioners in South Sardinia estimated a prevalence of adult MG of 35/100,000, including seronegative cases.[97] In Europe, a registry-based nationwide study in Sweden

(2006-2016), including all MG subtypes, reported peaks of incidence and prevalence of 29/million and 36/100,000, respectively.[109] Similar estimates of 31/million and 37/100,000 were recently reported in the United Stated (2016-2021), representing the highest values reported so far.[78] Our findings from 2010-2019 far exceed these estimates and the expected overall rate of rise of prevalence despite the selective inclusion of seropositive cases,[110] making Sardinia the region with highest MG risk reported so far.

After stratification by autoantibody, we found a high prevalence of both AChR MG and MuSK MG compared to antibody-specific data from Northern Europe.[104, 110] This is in keeping with prior studies suggesting MG might be more common at lower latitudes, although epidemiological data in Europe remain scarce.[14, 104, 110, 111]

The high MG risk in our study follows other highly prevalent immune-mediated disorders in Sardinia (*e.g.*, multiple sclerosis, autoimmune thyroiditis),[17, 23] suggesting a predisposition towards autoimmunity due to genetic and/or environmental factors. We found a high incidence of late-onset forms, especially among men, in line with recent findings from other cohorts.[24, 112, 113] The longevity of the Sardinian population might have further inflated the proportion of cases with onset ≥50 years of age, and contributed to the high disease prevalence as we were able to identify older MG cases diagnosed as early as 1998 and still alive at prevalence day.

Our second study is limited by the retrospective design. Our findings are generalizable to the entire regional population but not to other Italian regions owing to important genetic and environmental differences.[106, 114] The real MG incidence and prevalence in Sardinia are likely to be higher as we only included cases with AChR-IgG and MuSK-IgG positivity, and patients with available clinical details to avoid inclusion of rare patients with false antibody positivity.[115] Patients with lipoprotein-related protein 4 (LRP4)-IgG or other less common antibodies were not included as testing for these

antibodies is not routinary offered in Sardinia, and the optimal assays for their detection remain debated.[116] Furthermore, antibody positivity was determined by RIA, which remains the gold standard for MG diagnosis but has lower sensitivity compared to cell-based assays.[117, 118] Future studies may clarify the exact determinants of MG risk in Sardinia.

In **conclusion**, findings from this project highlight that:

1. Sardinia is a region at high risk for certain autoimmune neurologic disorders (e.g., MG), while other diseases like NMOSD have a risk that is comparable to that of other predominantly Caucasian populations (disease-specific risk).
2. MG cannot be defined a rare disease in Sardinia. Based on our findings we can estimate that approximately 800-1000 MG patients or more live in Sardinia, and deserve adequate healthcare support.
3. The high risk of demyelinating CNS disorders previously documented in Sardinia is mainly driven by MS, while the relative contribution of rare antibody-mediated forms like NMOSD seems marginal.

Our project represents the starting point for an extensive characterization of many other antibody-associated neurologic disorders in Sardinia and better understanding of these entities in the future.

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