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Increasing Incidence of Multiple Sclerosis in the Province of Sassari, Northern Sardinia

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Abstract
Sardinia is a high-risk area for multiple sclerosis (MS), with prevalence rates of 150 per 100,000 population. The study included 689 MS patients (female-male ratio 2.6) with disease onset between 1965 and 1999 in the province of Sassari. The mean annual incidence rate increased significantly from 1.1 per 100,000 population in 1965–1969 to 5.8 in 1995–1999, with no significant difference for gender and province sub-areas. The mean age at onset increased significantly during the same period from 25.7 to 30.6 years, while the proportion of patients with progressive initial course declined over time. The marked increase of MS incidence and the change of MS clinical phenotype over time cannot be explained by ascertainment bias only, thus pointing to a corresponding change in the distribution of exogenous risk factors in this highly genetically stable population.

Introduction

Previous population-based prevalence studies have shown that Sardinia is a high-risk area for multiple sclerosis (MS), with prevalence rates of 150 per 100,000 [1]. This is 2- to 3-fold higher than in other Italian or Caucasian populations and contrary to the latitude gradient theory [2]. The prevalence trend has increased in the past 3 decades [3–5]. The aim of this population-based study was to analyze the MS incidence trend in northern Sardinia by gender, initial clinical course, age of onset and areas of the province as identified in linguistic studies [6].

Material and Methods

Study Area

The study population is the province of Sassari, northern Sardinia (fig. 1), an area of 7,520 km^2 between the latitudes of 40°30′ N and 41° N and encompassing 90 municipalities. The total population in 2001 was 453,628 [7]. The population of the province of Sassari increased during the past 30 years from 381,191 to 453,628. Migration flow has been modest: in 1995, 1.7% of the total population was registered as having migrated from other provinces or countries, whereas 1.6% had moved away from the study area [8]. The proportion of residents born outside the province of Sassari is negligible and the migration to the study area is mostly from other provinces of Sardinia. The ethnic composition of the study population is therefore assumed to be fairly homogeneous, as it almost completely consists of native born individuals. Low immigration has led to the differentiation and, to a certain extent, to the isolation of geographical areas with specific historical, linguistic and cultural patterns, with in-breeding playing a role in most inland communities [9]. Specifically, seven ethnically homogeneous areas have been characterized for the province of Sassari (fig. 1): Sassarese (1), Gallurese (2), Northern Logudorese (3), Eastern Logudorese (4), Southern Logudorese (5), Goceano (6) and Algherese (Catalan) (22) [6].

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Fig. 1. Map showing the linguistic areas in the province of Sassari, northern Sardinia [6]: Sassarese (1), Gallurese (2), Northern Logudorese (3), Eastern Logudorese (4), Southern Logudorese (5), Goceano (6) and Algherese (Catalan) (22).

Patients

Cases were identified using the MS case registry established at the Institute of Clinical Neurology, University Hospital of Sassari, the primary referral center for MS patients in the province. A 'spider' kind of population based survey was used for case ascertainment and registry enrollment [2]. Because of the organization of neurological health facilities in the province territory, MS patients are caught in a network of peripheral health operators who refer to the Sassari MS center, where the registry is periodically updated and the access to medical records easy over time [1, 10]. The patients were diagnosed according to the Poser et al. [11] criteria, and other autoimmune and/or immune-mediated diseases and infectious diseases of the central nervous system were excluded by means of neurological history and examination and by laboratory tests and neuroimaging. If they had been diagnosed in another health system, MS patients underwent examination by trained institute neurologists, and their medical history was collected with the contribution of their closest relatives after informed consent. Information on patients' birth date and place, residence at clinical onset of disease, date and symptoms of clinical onset [12], date of diagnosis and disease classification were registered. All symptoms experienced within 3 months after the first symptom were regarded as symptoms at onset.

Incidence was studied from January 1, 1965 to December 31, 1999. An incident case was defined as any individual who first experienced symptoms later related to MS [12] while residing in the province of Sassari. The initial clinical course was retrospectively categorized into relapsing remitting (RR) course at onset or progressive course at onset.

Table 1. Crude (95% CI) and age-standardized average annual incidence rates per 100,000 population for MS for both sexes in the province of Sassari in 5-year intervals from 1965 to 1999

Period	n	Crude rate per 100,000	95% confidence interval	Age-standardized rate per 100,000
1965–1969	23	1.2	0.8–1.8	1.1
1970–1974	46	2.3	1.6–3.1	2.2
1975–1979	83	4.0	3.1–4.9	3.8
1980–1984	107	4.9	4.0–6.0	4.6
1985–1989	144	6.5	5.4–7.6	6.0
1990–1994	147	6.5	5.4–7.6	6.1
1995–1999	139	6.1	5.1–7.2	5.8
1965–1999	689	4.6	4.2–4.9	4.4

Statistical Analysis

The incidence rates were calculated using data from the census in 1971, 1981, 1991 and 2001. Age standardization was computed by adjusting for the general population of Italy as of the 2001 census [12]. The time period of onset 1965–1999 was divided into seven 5-year intervals in this analysis. A χ^2 test was applied to test any difference in incidence over time between gender, initial clinical course, onset symptoms and areas of onset, again using the seven 5-year intervals. Eastern Logudorese (4), Southern Logudorese (5), and Goceano (6) were aggregated to achieve sufficient population sizes (fig. 1).

Results

A total of 689 patients with onset of disease from 1965 to 1999 within the province of Sassari were included in the analyses: 496 women and 193 men, giving a female–male ratio of 2.6. The mean annual crude incidence rate for the whole province was 4.6 per 100,000 population: 6.5 for women and 2.6 for men. The overall age-adjusted incidence rate was 4.4. The age-adjusted incidence rate increased markedly and significantly from the beginning of the study period, from 1.1 per 100,000 population in 1965–1969 and 2.2 in 1970–1974 to a rather stable rate of about 6 for the last three 5-year periods, 1985–1999 (table 1). The trend did not differ significantly between genders during the study period (χ^2 test, table 2).

The crude incidence rate differed significantly between the linguistic areas during the whole study period (p =

Table 2. Gender-specific age-standardized average annual incidence rates per 100,000 population for MS in the province of Sassari, Italy in 5-year intervals from 1965 to 1999

	Men		Women		Female/ male ratio
	n	age-adjusted rate	n	age-adjusted rate	
1965–1969	9	0.9	14	1.3	1.6
1970–1974	17	1.8	29	2.7	1.7
1975–1979	20	2.0	63	5.4	3.2
1980–1984	29	2.6	78	6.5	2.7
1985–1989	35	3.2	109	8.7	3.1
1990–1994	39	3.4	108	8.7	2.8
1995–1999	44	3.8	95	7.6	2.2
1965–1999	193	2.6	496	6.1	2.6

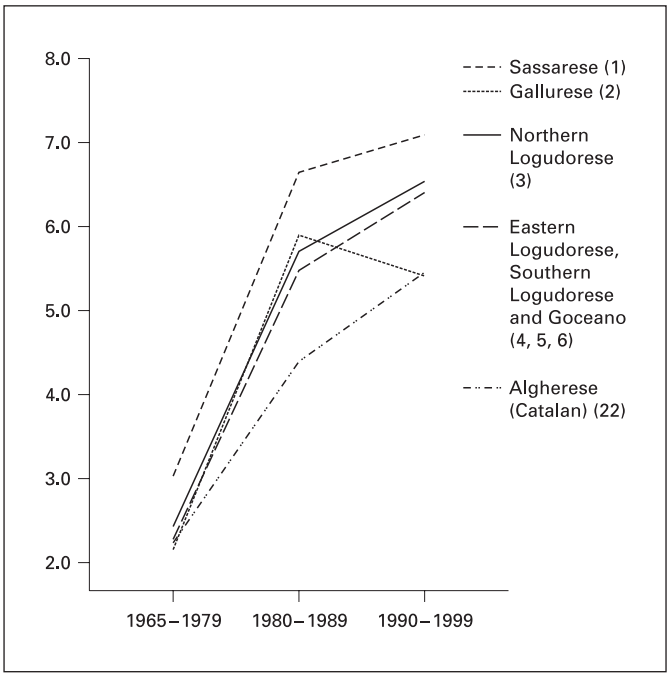


Fig. 2. Incidence rates according to areas through three time periods.

Table 3. Mean crude incidence rate of MS for the whole period according to linguistic areas per 100,000 population for MS for 1965–1999

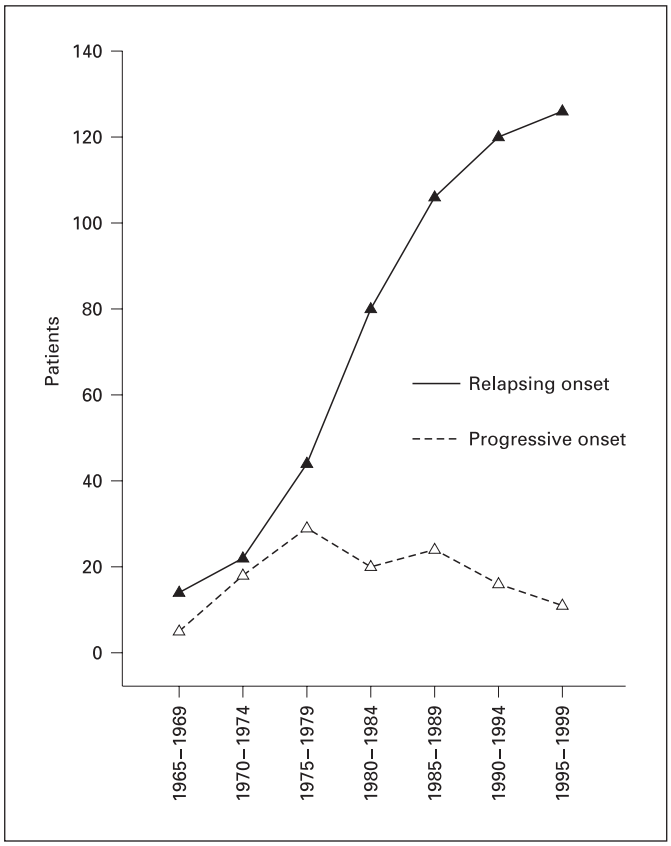
Linguistic area	Average population for 1965–1999	n	Crude rate	95% CI
Sassarese (1)	157,456	295	5.4	4.8–6.0
Gallurese (2)	71,854	108	4.3	3.5–5.2
Northern Logudorese (3)	62,980	100	4.5	3.7–5.5
Eastern Logudorese (4), Southern Logudorese (5) and Goceano (6)	96,786	130	3.8	3.2–4.5
Algherese (Catalan) (22)	35,376	56	4.5	3.4–5.9
Total area	425,442	689	4.6	4.3–5.0

0.02, χ^2 test), with Sassarese showing the highest rate of 5.4 and the Eastern and Southern Logudorese and Goceano areas with the lowest rate of 3.8 (table 3). The incidence increased over time in all areas, and this time trend did not differ significantly (fig. 2).

The clinical course at onset, evaluated retrospectively, was known for 635 cases, with 512 patients (81%) with RR and 123 patients with a progressive course at onset. There was a significantly higher increasing trend among the group with RR course at onset than among those with progressive one ($p < 0.001$, χ^2 test) (fig. 3). In fact, the proportion of patients with RR onset increased from 61% during 1965–1979, to 90% during 1985–1999.

Table 4 presents the distribution of clinical manifestations at onset for the whole study period. The most frequent symptoms at onset were sensory (40% of the patients), pyramidal (22%) and visual manifestations (22%). The distribution of manifestations at onset did not differ significantly between 1965–1979, 1980–1989 and 1990–1999 (χ^2 test, data not shown).

The mean age at onset for the whole study period was 28.6 years (95% confidence interval (CI): 27.9–29.2). The mean age at onset did not differ significantly (t test) between men (29.4 years) and women (28.2 years). Patients with RR course at onset had no significant difference in age at onset from patients with progressive course at onset



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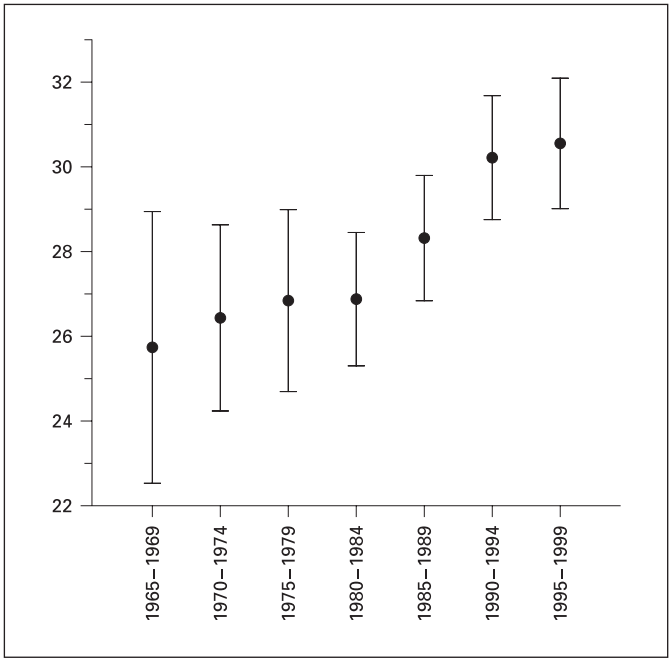

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Fig. 3. Distribution of number of patients according to type of initial clinical course through the seven 5-year periods.
Fig. 4. Distribution of mean age at onset (95% CI) over the seven 5-year time periods.

(t test): 28.0 vs. 29.3 years. The percentage of patients with late onset (45 years and older) was 4.8 for the whole study population.

The mean age at onset increased steadily and significantly from 25.7 years in 1965–1969 to 30.6 years in 1995–1999 (fig. 4). Since age at onset and clinical course are related and since the distribution of both these clinical variables changed over time, we performed an analysis of variance with age at onset as the dependent variable and time period (using the seven 5-year intervals) and course of disease as fixed factors. Since the shift towards more cases with initial relapsing course during the last periods is expected to be associated with a lower age at onset, the estimated increase in age at onset over time was even more marked in this model. Likewise, the effect of initial course on age at onset was statistically significant in this model, with an estimated difference of 2.6 years as compared with the observed difference of 1.3 years between patients with progressive onset and patients with relapsing onset.

The time lag between clinical onset and diagnosis decreased significantly from 13.0 years in 1965–1969 to 0.9 years in 1995–1999.

Table 4. Distribution of MS symptoms at onset for the whole study period

Type of symptom	n	%
Pyramidal	77	22
Cerebellar	25	7
Brainstem	59	17
Bowel and bladder	6	2
Visual	76	22
Sensory	138	40
Other	18	5

Discussion

The results of the present study show that the increased temporal trend in MS prevalence previously observed for the province of Sassari [1] was caused by a corresponding rise in incidence. The incidence rate is not influenced by any improved survival. Further, even if the reduced time lag between clinical onset and diagnosis indicates an in-

tensified case-finding over time, the patients diagnosed in more recent periods due to this, still have been assigned their true year of clinical onset. The improved case-ascertainment can only influence the incidence if patients in the early periods have died before being diagnosed. This is unlikely to have occurred with this chronic disease over the relatively short time period and the survey design with multiple assessments. Also, although improvement of diagnostic accuracy could have been responsible for an earlier diagnosis and subsequent report of a greater number of cases, when comparing our data with those from a northern Italian health district with better technological diagnostic facilities, such as MRI, no differences were detected in the temporal trend of the time lag elapsing from clinical onset to diagnosis [1, 14]. The increase of MS in northern Sardinia therefore most likely reflects a corresponding change in underlying risk factors for the disease.

The incidence rates were stable during the last three 5-year periods. Nevertheless, because incidence studies are conducted on patients who have received a diagnosis, true incidence rates from the more recent period are likely to be underestimated given that patients have not yet been registered [15].

Clinical characteristics at disease onset deviated in two ways from an evenly distributed increase of incidence rates. Firstly, the increase mainly applied to patients with a RR course at onset. This has also been found in other studies showing an increased incidence of MS and is sometimes ascribed to recall bias because patients fail to report their first episode(s) in the most remote years of the study period. In fact, some of these patients defined as having had a progressive onset in the early years may have instead been misclassified. Had this bias occurred, age at onset would also have been affected towards higher estimates for these cases.

Due to the retrospective nature of the survey, a bias toward identifying vague symptoms as attributable to MS might have been induced. Should vague symptoms at onset be missed for the patients with onset in the earliest part of the study period, then the estimated date of onset would have been later than the real one. The lower age at onset for these cases does not argue for such a bias.

Nevertheless, the other significant finding was, in fact, a younger age at disease onset for the first time intervals. The age at onset increased over the whole study period. This argues against the change of course over time due to recall bias and shows that the MS clinical phenotype must have changed over time. This might indicate a change in the exposure to one or more exogenous causative factors for MS over time, since the population of Sardinia is genetically stable.

A higher age at onset for the patients with initial progressive course is often reported [16–19]. The analysis of variance model that took into consideration this relationship showed that if the distribution of type of initial course had been stable over time, there would have been an even stronger increasing age at onset.

An anticipation of age at onset in MS among Sardinians with a mean age at onset of 41 years for patients born between 1913 and 1939, to 22 years for those born after 1970 has been recently reported by Cocco et al. [20]. This is in clear contrast with our observations. The previous study was not population-based since it included consecutive patients referred to the clinic from an undefined area of the island. It is therefore likely that a selection bias was introduced by excluding patients with very benign or very severe MS.

Furthermore, in the absence of a long maintained registry system, the date of onset was estimated retrospectively at time of the study, thus challenging the validity of these estimates for the oldest cohorts. By failing to report the first episode, biased estimates towards older age at onset for these cohorts are likely to have been reported. Lastly, the analysis was performed by decade of birth (<1940, 1940–1949, to >1970) leading to an obvious underestimation of age at onset for the youngest cohorts in which late onset MS may not have occurred yet. The authors made some kind of adjustment for this, but failed to present more reliable data, e.g. by analyzing the data according to time-periods rather than birth cohorts. In our opinion, the methodological approaches used in this study have lead to a biased estimate of the trend of age at onset. This probably accounts for the different result between the study of Cocco et al. [20] and the present study.

The incidence rates differed significantly between the linguistic areas. The westernmost areas (Sassarese, Northern Logudorese and Algherese) had higher mean rates than the easternmost ones. This is in agreement with that reported from a cluster analysis of the distribution of prevalence rates in the same study area, which revealed spatial clustering of MS in the western areas of the province [10]. Because of the methodology adopted for the current study, we feel reasonably confident in ruling out that different rates in urban and rural areas, as well as in areas bordering with the Sassarese domain, might be due to biased case ascertainment from the different parts of the province.

Nevertheless, as a parallel increase in the incidence rate was reported for all areas over time, differences in

genetic factors or in gene-environment interaction effects can be suggested to account for the geographical variation. In fact, evidence demonstrates that the distribution of some gene frequencies [21] in northern Sardinia clearly differentiates the easternmost area (Gallurese) from the Sassarese and Northern Logudorese.

Interestingly, an evenly distributed increase in incidence among Sardinians over comparable periods of time has also been reported for other immune-mediated disorders sharing specific immunogenetic features with Sardinian MS, such as HLA-DR-DQ haplotypes (DRB1*0301-DQA1*0501-DQB1*0201) in juvenile diabetes [22–24]. Improved survival rates, reduced stillbirth, perinatal and first-year mortality rate, a more accurate case ascertainment and emigration could not explain juvenile diabetes incremental trends [22]. The rather homogeneous geographical variation in the risk of IDDM throughout the island coupled with the marked general increase in incidence was pointing to an environmental factor relatively uniformly distributed in the territory that has rather recently changed its exertion [23]. This interpretation appears to be the most reasonable in also explaining the steady increasing of MS incidence over time. Moreover, the change in the MS clinical phenotype with an increasing age at onset and a decreasing proportion of patients with progressive initial course argues further for a corresponding change in an environmental etiological factor in this genetically stable population.

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