

Research Article

Antibody-Positive Autoimmune Encephalitis and Paraneoplastic Neurological Syndrome: Epidemiology and Outcome of Neuronal Antibody Testing in Sweden

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Objective. To estimate the 5-year incidence rate of autoimmune encephalitis (AE) and paraneoplastic neurological syndrome (PNS) in Sweden. **Methods.** All patients who were tested for a neuronal antibody in Sweden between 2015 and 2019 were included. Patients in Healthcare region Mid Sweden (population 2.1 million) were invited to participate in a case ascertainment substudy. AE and PNS cases were defined using established diagnostic criteria. Crude and age-adjusted incidence rates of AE and PNS in Healthcare region Mid Sweden were estimated. **Results.** The number of tests for neuronal antibodies in Sweden increased between 2015 and 2019 from 1867 to 2505 (serum) and 863 to 1376 (CSF) per annum. The frequencies of positive results were stable over the entire study period, and the mean value was 6.1% for serum (CI95% 5.5–6.7) and 4.8% for CSF (CI95% 4.0–5.6). In total, 125 patients tested positive for neuronal antibodies in Healthcare region Mid Sweden between 2015 and 2019. Of these, 94 were included, and after case ascertainment, thirty-one cases of definite AE or PNS could be identified. The 5-year incidence rate of AE and PNS was 3.0 per million person-years (95% CI 1.9–4.1). The yearly incidence rates increased in the study period, from 1.5 per million person-years in 2015 (95% CI 0.0–3.2) to 4.3 per million person-years in 2019 (95% CI 1.5–7.1). **Conclusions.** In this first epidemiological study of AE and PNS in Sweden, the number of cases doubled from 2015 to 2019. This likely reflects increased availability of testing and awareness of these conditions.

1. Introduction

Autoimmune encephalitis (AE) and paraneoplastic neurological syndrome (PNS) are rare immune-mediated disorders of the nervous system. Correct diagnosis relies on testing of autoantibodies targeting neuronal antigens (neuronal antibodies) and clinical diagnostic criteria [1, 2]. Epidemiological studies of AE and PNS have demonstrated an increase in incidence in the last decade, reflecting heightened awareness and the discovery of novel neuronal antibodies [3–9]. As early treatment leads to better outcomes [10–12], a swift and correct diagnosis is crucial. With neuronal antibody testing being more widely available in routine

healthcare, the demands on physicians to properly interpret the results in the clinical setting have increased. Many routine clinical laboratories use commercial kits for simplicity, which may affect diagnostic accuracy [13–16].

This study describes the epidemiology of AE and PNS in the second largest healthcare region of Sweden and investigates the use of neuronal antibody testing and some of its potential pitfalls in a real-world setting with routine diagnostics. The 5-year incidence rate of AE and PNS in Healthcare region Mid Sweden between 2015 and 2019 was estimated along with yearly crude and age-adjusted incidence rates. We also report the positivity rate of neuronal antibody testing in all of Sweden.

2. Methods

2.1. Study Population. Sweden had a population of 10,379,295 in 2020, and one in five was 65 years or older (2020, Statistics Sweden). The Swedish healthcare system is tax-funded, decentralized, and run by healthcare regions. There are six healthcare regions, the second largest being Healthcare region Mid Sweden with 2,128,642 inhabitants (2020, Statistics Sweden). Healthcare region Mid Sweden is serviced by two university hospitals (Uppsala University Hospital, Örebro University Hospital), five county hospitals, and eighteen rural hospitals. Rural hospitals refer patients to county or university hospitals in the same healthcare region.

2.2. Data on Antibody Analyses. Analysis of neuronal antibodies is performed in five laboratories in Sweden. Four of them are run by university hospitals (Uppsala University Hospital, Karolinska University Laboratory, Sahlgrenska University Hospital and Skåne University Hospital), and one is run by a private enterprise (Wieslab AB). Results from all patients tested for neuronal antibodies between 2015 and 2019 in these five laboratories were retrieved, hence, providing national coverage. The analyzed samples were serum and/or cerebrospinal fluid (CSF). Data on the results of analysis for antibodies against AMPA 1/2, Amphiphysin, CASPR2, CV2/CRMP5, DPPX, GABA-B, GAD65, Hu (ANNA-1), IgLON5, LGI1, Ma2/Ta, NMDAR, PCA-2, Tr, Ri (ANNA-2), SOX1 (AGNA), Yo (PCA-1), and Zic4 were retrieved. Furthermore, data on test date, the healthcare region requesting the test as well as the sex and age of the patients tested was collected. Patients with a positive test result requested by a healthcare provider in the Healthcare region Mid Sweden were contacted and invited to participate in the case ascertainment part of the study. No effort was made to adjust for potential selection bias caused by the exclusion of patients who declined to participate.

2.3. Antibody Testing. All five laboratories used an indirect fixed cell-based immunofluorescence assay (Euroimmun AG, Lübeck, Germany) to detect antibodies targeting extracellular antigens. Only one laboratory used immunofluorescence on neuronal tissues as a complementary screening method.

To detect antibodies targeting intracellular antigens, a combination of indirect immunofluorescence and immunoblot was most often used (antibodies needed to be detected by both methods to be considered a positive result), although sometimes, only immunoblot was used. Most of the laboratories also provided quantification of GAD65-antibodies using enzyme-linked immunosorbent assay (ELISA). GAD65 antibodies > 2000 IU/ml detected by ELISA in serum, or detected at all in CSF, was considered a positive test result and included in this study (except for from one laboratory that did not provide quantification of serum samples where all positive serum samples were included for case ascertainment).

During the 5-year period included in this study, some laboratories changed their repertoire of tests and their test methods (the appendix).

2.4. Case Ascertainment. Medical records of patients in Healthcare region Mid Sweden were reviewed to obtain clinical, laboratory, and imaging data. The following diagnostic criteria were used for case ascertainment: (1) definite PNS (≥ 8 points) according to the PNS-Care Score proposed by Graus et al. [2] or (2) definite autoimmune encephalitis (definite AE) according to Graus et al. [1] or (3) definite anti-NMDAR encephalitis according to Graus et al. [1].

2.5. Statistical Analysis. The 5-year incidence rate of AE and PNS in Healthcare region Mid Sweden between 2015 and 2019 was calculated as the number of new ascertained cases divided by the number of person-years contributed by the average population of that time period. Incidence rates of subgroups, AE (definite AE and definite anti-NMDAR encephalitis), PNS (definite PNS), anti-NMDAR encephalitis, and anti-LGI1 encephalitis, were calculated using the same method. Patients who fulfilled diagnostic criteria of both definite AE or anti-NMDAR encephalitis and definite PNS were included in both subgroups for the separate incidence rates but only counted as one case in the composite incidence rate for both AE and PNS. Crude yearly incidence rates for AE and PNS were calculated as the number of new ascertained cases each year divided by the average population of that year. Incidence rates were standardized directly to the European standard population 2013 [17]. Population data from Statistics Sweden (SCB) were used. Confidence intervals (95% CI) were calculated assuming a Poisson distribution and using a normal approximation. The positivity rate for neuronal antibody testing in Sweden between 2015 and 2019 was calculated as the number of patients with a positive test result divided by the total number of patients tested each year, for serum and CSF samples, respectively. The ratio of failed case ascertainment was calculated as the number of positive tests where case ascertainment failed divided by the total number of patients included in the case ascertainment. Estimated incidence rates of AE and PNS, anti-NMDAR encephalitis, and anti-LGI1 encephalitis in the entire Swedish population 2015–2019 were calculated. Cases were defined as the number of patients who tested positive for any neuronal antibody, anti-NMDAR, or anti-LGI1 antibodies, adjusted by the ratio of failed case ascertainment acquired in Healthcare region Mid Sweden. The cases were then divided by the number of person-years contributed by the average Swedish population during that time period. Descriptive statistics are presented, and parametric variables are reported as mean \pm SD. Statistical analysis was performed using GraphPad Prism version 9.3.1 for Mac (GraphPad software, La Jolla, CA, USA; <http://www.graphpad.com>).

2.6. Standard Protocol Approvals, Registrations, and Patient Consent. This study was approved by the Swedish Ethical Review Authority (Dnr: 2019-03068). Signed informed consent was required for inclusion in the case ascertainment part of the study. In line with the approved ethical application, consent was presumed for deceased patients. This study was registered at Clinicaltrials.gov (Identifier: NCT04708626).

3. Results

3.1. Neuronal Antibody Test Results in Sweden 2015–2019. In all of Sweden, 525 patients (49% female) tested positive for a neuronal antibody between 2015 and 2019 (serum and/or CSF, duplicates removed, Figure 1). Neuronal antibodies were detected in serum only in 388 patients, in CSF only in 49 patients, and in both serum and CSF in 88 patients. The most frequently detected antibody in serum was anti-GAD65 and in CSF anti-NMDAR (Figure 2). Among patients with a positive test result, 125 belonged to Healthcare region Mid Sweden. The case ascertainment included 94 patients (45% female, age 61 ± 18 , 19% with anti-NMDAR antibodies and 14% with GAD65 antibodies). Thirty-one patients were excluded from further analysis due to lack of consent, incomplete personal data, diagnosis before 2015, or missing medical records (39% female, age 49 ± 23 , GAD65 antibodies 26%, and anti-NMDAR antibodies 16%).

3.2. Case Ascertainment. Among the 94 included patients, thirty-one fulfilled the diagnostic criteria for definite AE, definite anti-NMDAR encephalitis, or definite PNS. Eight patients fulfilled criteria for both definite AE and definite PNS concurrently: two cases of anti-GABA-B receptor encephalitis, two cases of anti-Ma2/Ta encephalitis, and four patients with anti-Hu. One patient with definite anti-NMDAR encephalitis also fulfilled criteria for definite PNS. Anti-NMDAR encephalitis and anti-LGI1 encephalitis were the most prevalent subgroups of AE during this time period (Figure 3). Patients with anti-NMDAR encephalitis were predominantly female (83%; age 45 ± 22), whereas patients with anti-LGI1 encephalitis were mostly male (67%) and older (age 73 ± 5.2). Among patients with definite PNS, there was a female majority (67%; age 69 ± 8.7). Five patients tested positive for more than one type of neuronal antibody, for example, one 67-year-old man with anti-Hu paraneoplastic encephalitis had both Hu and Zic4 antibodies in serum and CSF. A 53-year-old woman had LGI1 and CASPR2 antibodies detected in her serum concurrently and a diagnosis of Morvan's syndrome with a malignant thymoma.

3.3. Incidence Rates of AE and PNS. The crude incidence rate of AE and PNS in the Healthcare region Mid Sweden 2015–2019 was 3.0 per million person-years (CI95% 1.9–4.1). The yearly crude incidence rates of AE and PNS increased in the 5-year period under study, from 1.5 per million person-years in 2015 (CI95% 0.0–3.2, average population of 2,022,479) to 4.3 per million person-years in 2019 (CI95% 1.2–7.1, average population of 2,111,058; Table 1).

3.4. Positivity Rate of Neuronal Antibody Testing. The number of patients tested for a neuronal antibody in the entire Swedish population was 1867 (serum) and 863 (CSF) in 2015 and increased to 2505 (serum, 34% increase) and 1376 (CSF, 60% increase) in 2019 (Figure 4). The mean proportion of patients with a positive serum sample over this 5-year period was 6.1% (CI95% 5.5–6.7), and the mean proportion of patients with a positive CSF sample was 4.8% (CI95% 4.0–5.6; Figure 4).

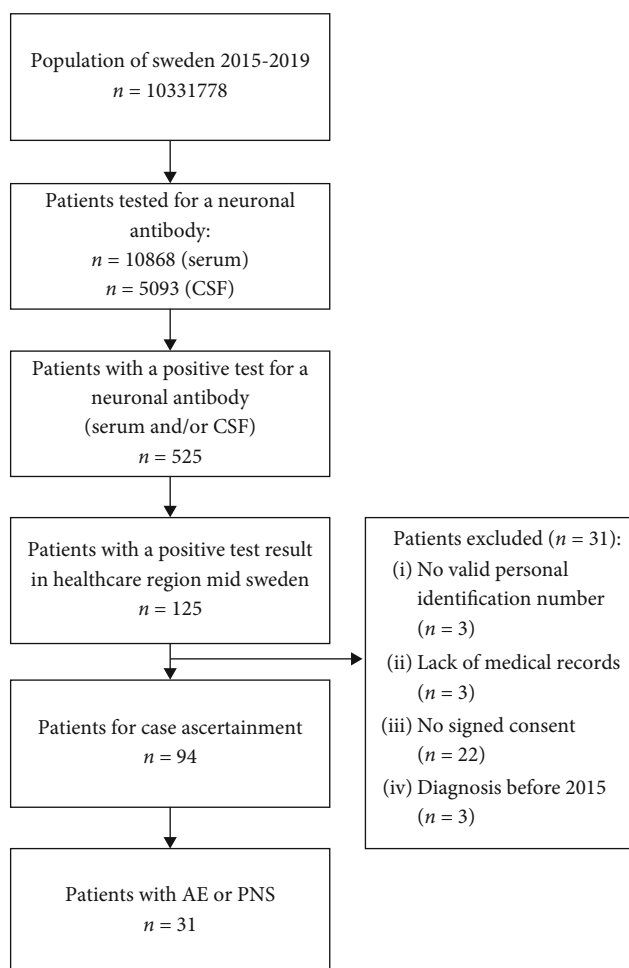


FIGURE 1: Flow chart of study design.

3.5. Patients Not Meeting Diagnostic Criteria. Case ascertainment failed in 63 of 94 included patients (67%). Patients who did not meet the diagnostic criteria of definite AE, definite anti-NMDAR encephalitis, or definite PNS had various diagnoses (Table 2). Notably, four of these patients had PNS according to their treating physician but did not fulfill diagnostic criteria of definite PNS; however, they did meet the criteria of probable PNS using the PNS-Care Score. AE was diagnosed in two patients who did not meet diagnostic criteria and autoimmune cerebellitis in another two patients. Anti-IgLON5 encephalopathy was diagnosed in one patient during the study period.

3.6. Estimated Incidence Rates of AE Subgroups in Sweden. In total, 525 patients in Sweden tested positive for a neuronal antibody in the study period. Assuming case ascertainment would fail in the same ratio as in the Healthcare region Mid Sweden (67%), the estimated incidence rate of AE and PNS would be 3.5 per million person-years (CI95% 2.9–4.0). All patients who tested positive for anti-LGI1 antibodies in the Healthcare region Mid Sweden met the diagnostic criteria of definite AE. In the entire Swedish population in 2015–2019, 41 patients tested positive for anti-LGI1 antibodies (68% male, age 65 ± 14), and assuming

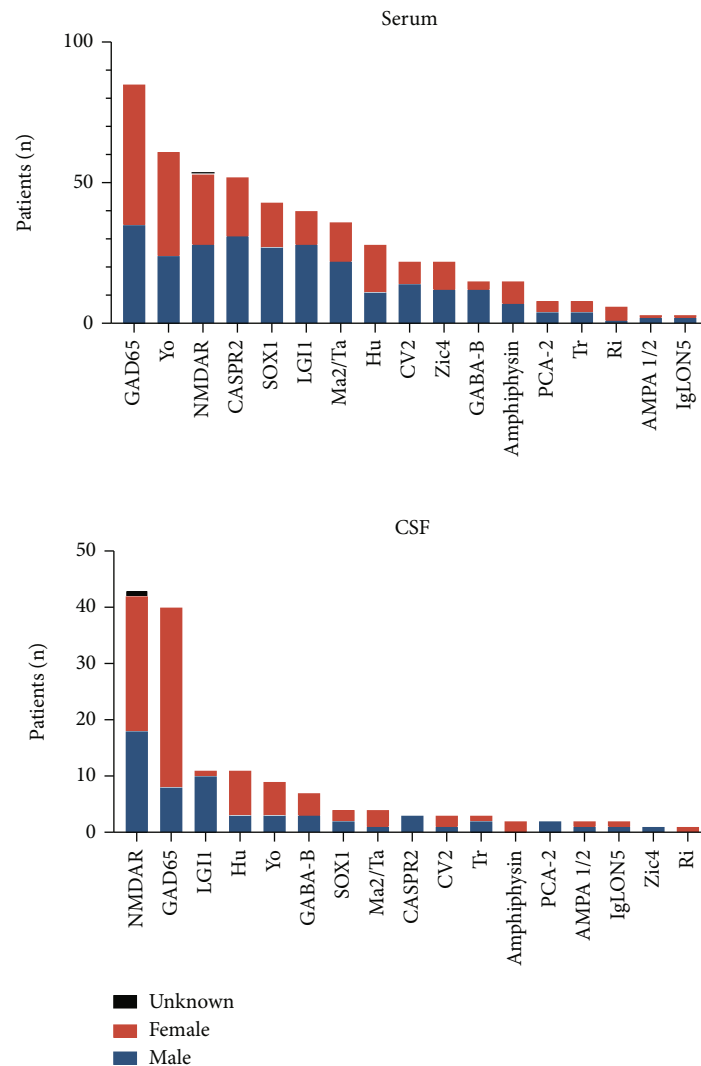


FIGURE 2: Antibody subgroups of patients who tested positive for a neuronal antibody in all of Sweden 2015–2019, serum and CSF displayed separately, and sex distribution shown. Patients who tested positive in both serum and CSF are represented in both graphs. Patients who tested positive for more than one type of antibody are represented in more than one column.

all these patients represented true cases, the estimated crude incidence rate of anti-LGI1 encephalitis would be 0.82 per million person-years (CI95% 0.57–1.1).

Among patients who tested positive for anti-NMDAR antibodies in Healthcare region Mid Sweden, 60% did not meet the diagnostic criteria of definite anti-NMDAR encephalitis. In Sweden, anti-NMDAR antibodies were detected in 75 patients (47% female, age 36 ± 24 (mean \pm SD)) during the years 2015–2019. The estimated crude incidence rate of anti-NMDAR encephalitis in Sweden 2015–2019 would be 0.60 per million person-years (CI95% 0.38–0.81) assuming that case ascertainment would fail in the same ratio (60%) in the entire Swedish population.

4. Discussion

This is the first country-wide epidemiological study of AE and PNS in Sweden and an analysis of challenges associated with neuronal antibody testing. The crude incidence rate of

AE and PNS in Healthcare region Mid Sweden was 3.0 per million person-years (CI95% 1.9–4.1), with increasing yearly crude incidence rates during the study period. The incidence rate of AE and PNS was found to be 3.2 per million person-years in France between 2016 and 2018, but with a distinctly higher incidence rate (7.5 per million person-years) in the Rhône-Ain-Isère region which lies close to the National Reference Center of France [4]. In the same study, the incidence rate of antibody-positive AE was 1.8 per million person-years in the whole of France and 3.6 per million person-years in the Rhône-Ain-Isère region, which puts our crude estimate of 2.4 per million person-years somewhere in between.

Anti-LGI1 encephalitis had a crude incidence rate of 0.58 per million person-years in Healthcare region Mid Sweden 2015–2019, very similar to the French estimate of 0.6 per million person-years [4], although slightly lower than previous estimates in Italian and Dutch populations (0.84 and 0.83 per million person-years) [7, 8]. Considering the

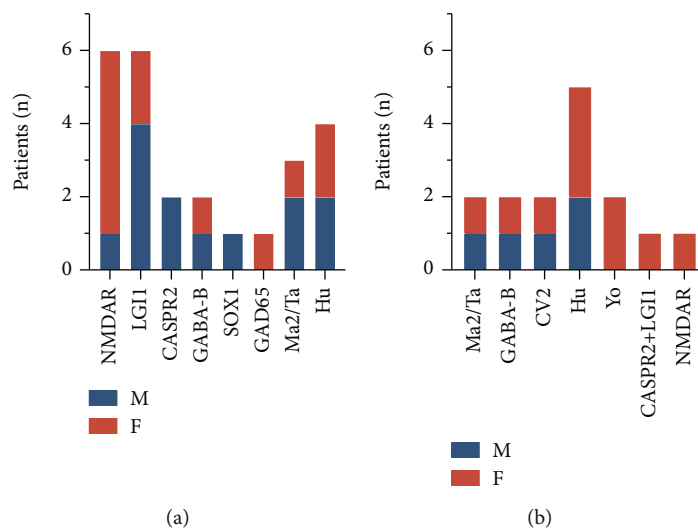


FIGURE 3: (a) Antibody subgroups of patients with definite AE or anti-NMDAR encephalitis in Healthcare region Mid Sweden 2015–2019. (b) Antibody subgroups of patients with definite PNS in Healthcare region Mid Sweden 2015–2019. Patients concurrently fulfilling diagnostic criteria of definite AE and PNS, or definite anti-NMDAR encephalitis and PNS are represented in both diagrams. AE: autoimmune encephalitis; PNS: paraneoplastic neurological syndrome; M: male; F: female.

TABLE 1: Crude and age-adjusted incidence rates of AE and PNS in Healthcare region Mid Sweden 2015–2019.

Subgroup and time period	Crude incidence (CI95%) [†]	Age-adjusted incidence (CI95%) [‡]
AE + PNS 2015–2019	3.0 (1.9–4.1)	2.9 (1.8–3.8)
AE + PNS 2015	1.5 (0.0–3.2)	1.4 (0.0–2.9)
AE + PNS 2016	2.4 (0.30–4.6)	2.2 (0.027–4.2)
AE + PNS 2017	2.9 (0.58–5.2)	2.5 (0.30–4.6)
AE + PNS 2018	3.8 (1.2–6.5)	3.8 (1.2–6.5)
AE + PNS 2019	4.3 (1.5–7.1)	3.8 (1.3–6.3)
AE 2015–2019 [§]	2.4 (1.5–3.4)	2.4 (1.4–3.2)
PNS 2015–2019	1.5 (0.72–2.2)	1.3 (0.66–2.0)
Anti-NMDAR encephalitis 2015–2019	0.58 (0.12–1.1)	0.58 (0.12–1.0)
Anti-LGI1 encephalitis 2015–2019	0.58 (0.12–1.1)	0.53 (0.11–0.95)

[†]Crude incidence rate per million person-years. [‡]Age-adjusted incidence rates per million person-years standardized to the 2013 European Standard population. [§]AE: definite AE and definite anti-NMDA receptor encephalitis. The population of Healthcare region Mid Sweden contributed with 10,331,778 person-years between 2015 and 2019. AE: autoimmune encephalitis; PNS: paraneoplastic neurological syndrome.

positive test results for anti-LGI1 antibodies in the entire Swedish population and assuming that all represent true cases gave us an estimated incidence rate of 0.82 per million person-years. Anti-NMDAR encephalitis had an incidence rate of 0.58 per million person-years in our study, compared with 0.89 per million person-years 2009–2018 in Denmark [5]. Both the age and sex distribution of anti-NMDAR encephalitis in our study differed from previous studies [18], with surprisingly many older patients (33% >45 years) and males. There were only two children (age ≤ 19) with anti-NMDAR antibodies in Healthcare region Mid Sweden 2015–2019. One 17-year-old patient with definite anti-NMDAR encephalitis was included in this study. The second child was excluded due to lack of written consent from parents. With the inclusion of only one child, the incidence rate of anti-NMDAR encephalitis in children (0–19 years) was 0.42 per million person-years in our study population, lower

than in a Danish study (0.7 per million person-years) [19]. However, had both children been included, the incidence rate instead would have been 0.85 per million person-years (in children 0–19 years). At least in the case of anti-NMDAR encephalitis and anti-LGI1 encephalitis, the discrepancy between incidence rates in our and previous studies is most likely due to our study design and not true differences in incidence. The deviant age and sex distribution of anti-NMDAR encephalitis in our study population suggests that there is potential for more testing in children and females.

To our knowledge, this is the first epidemiological study in Europe where the PNS-Care Score [2] was used for case ascertainment of definite PNS, making comparisons with previous studies using the 2004 diagnostic criteria [20] potentially problematic. For example, a diagnosis of definite PNS according to the PNS-Care Score requires the presence

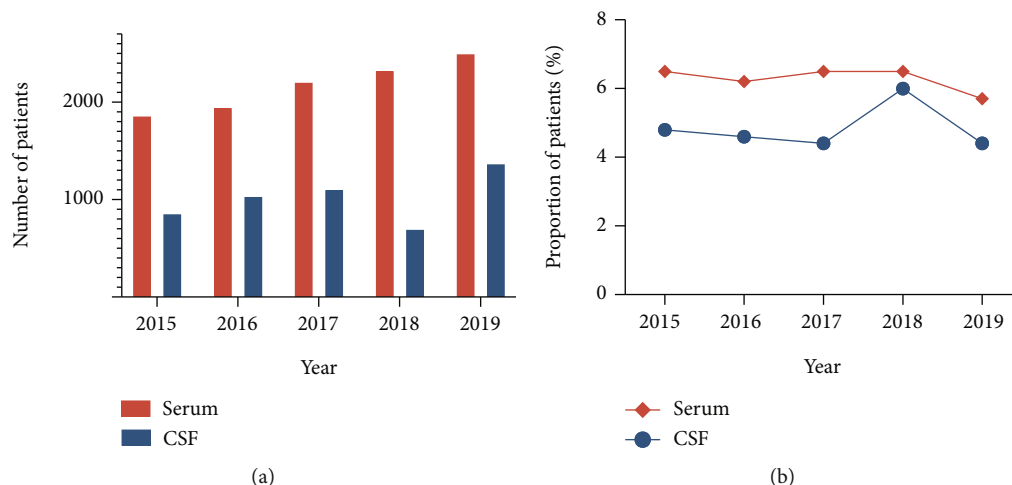


FIGURE 4: (a) The total number of patients tested for a neuronal antibody in Sweden between 2015 and 2019, serum and CSF samples presented separately. (b) The proportion of patients (%) tested for a neuronal antibody that had a positive test result, serum and CSF samples presented separately, between 2015 and 2019 in Sweden.

TABLE 2: Final diagnosis of patients not fulfilling diagnostic criteria of definite AE or definite PNS.

Diagnosis	Patients (<i>n</i>)	Antibodies
Probable PNS [†]	4	CV2, Amphiphysin, SOX1
Malignancy without PNS	4	Zic4, CASPR2, GAD65
Autoimmune CNS disease	5	
Autoimmune encephalitis [‡]	2	CASPR2, GAD65
IgLON5 encephalomyelitis	1	IgLON5
Autoimmune cerebellitis	2	GAD65
Stiff person syndrome	1	GAD65
CNS infection	5	CV2, Ma2/Ta, NMDAR, CASPR2, Yo
Demyelinating disorders of CNS	2	NMDAR, Zic4
Neuropathy	8	
AIDP	3	SOX1, Hu, CV2
CIDP	2	GAD65, Zic4
Other	3	Yo, GABA-B, SOX1
Neurosarcoidosis	1	SOX1
Movement disorders	9	
Cerebellar ataxia	4	SOX1, Ma2/Ta, Ri, NMDAR
Other	5	Amphiphysin, CASPR2, Yo, Zic4
Creutzfeldt Jakob's disease	1	NMDAR
Psychiatric diagnosis	4	NMDAR, Zic4, Ma2/Ta
Diabetes type 1	5	GAD65
Other	14	GAD65, Yo, CASPR2, CV2, SOX1, Zic4, NMDAR

The final diagnosis, according to treating physician, of patients who did not meet criteria for definite AE, definite anti-NMDAR encephalitis, or definite PNS but tested positive for a neuronal antibody 2015–2019 in Healthcare region Mid Sweden. There is no certain association between all diagnoses and antibodies presented in this table. [†]Probable PNS according to PNS-Care Score, diagnosed as PNS by treating physicians. [‡]Diagnosed as autoimmune encephalitis by treating physician but does not fulfill diagnostic criteria. Abbreviations: AE: autoimmune encephalitis; PNS: paraneoplastic neurological syndrome; AIDP: acute inflammatory demyelinating polyneuropathy; CIDP: chronic inflammatory demyelinating polyneuropathy.

of both neuronal antibodies and cancer consistent with the phenotype, whereas the old criteria required only one of the two. Our study estimated a crude incidence rate for definite PNS in Healthcare region Mid Sweden 2015–2019 to be

1.5 per million person-years, with age-adjusted incidence rate being 1.3 per million person-years (European standard population 2013). This is lower than an estimate of 8.9 per million person-years (standardized to the European

standard population 2013) from Northeastern Italy [9], which however included antibody-negative PNS (antibody-positive PNS constituted around 25% of cases which would yield an estimated incidence rate of 2.3 per million person-years). In the Rhône-Ain-Isère region, the incidence rate of antibody-positive PNS was 1.7 per million person-years [4]. In Olmsted County, USA, the incidence rate of PNS was 8 per million person-years 2003–2018 [21], but this study included both patients with myasthenia gravis and dermatomyositis. The American study calculated the PNS-Care Score of included cases, but only 8 of 28 fulfilled criteria of definite PNS, which would yield an incidence rate of 2.3 per million person-years. There are several explanations for our lower estimate other than our strict definition of cases. Our study did not include patients tested for VGCC-antibodies, hence excluding patients with Lambert Eaton myasthenic syndrome. The incidence of cancer (and consequently PNS) might also vary in different populations, e.g., Sweden has among the lowest incidences of lung cancer in Europe [22]. Future epidemiological studies of PNS using the PNS-Care Score are needed to evaluate the generalizability of our findings.

The positivity rate of neuronal antibody testing in Sweden was slightly lower at the end of the study period compared with at the start (5.7% vs. 6.5% serum, 4.4% vs. 4.8% CSF), and the total number of patients tested increased (34% increase of serum tests from 2015 to 2019, 60% increase of CSF tests). Previous studies in Germany and Italy have shown similar positivity rates of neuronal antibody testing (5.3% and 7.8% positive samples) [8, 23]. Case ascertainment failed in almost the same ratio 2015 as 2019 (70% vs. 65%) suggesting that increased testing was adequate. It is possible that further increased testing would yield even more cases of AE and PNS. However, the high proportion of patients with a different final diagnosis present difficulties for physicians as a test result incompatible with the clinical presentation might cause confusion about treatment and the need for future cancer screening. Improved selection of patients may lead to increased diagnostic accuracy. The use of scores such as the APE [2] score could be a way to increase the pretest probability of a true positive outcome [24].

The samples from Healthcare region Mid Sweden were almost exclusively analyzed by one of two different laboratories (93 of 94 patients). One laboratory relied on immunoblot for detection of classic onconeural antibodies (Ma2/Ta, CV2, Hu, Ri, Yo, and Amphiphysin) for most of the study period; the other used a combination of indirect immunofluorescence and immunoblot. The ratio of failed case ascertainment differed between the two laboratories regarding classic onconeural antibodies. The ratio was 81% among the samples from the laboratory using only a commercial line blot, whereas the ratio was 63% in the laboratory using two independent methods, consistent with previous publications on diagnostic accuracy [13–15]. This difference contributed to the overall high proportion of patients not meeting diagnostic criteria of definite AE or definite PNS in our study, together with our inclusion of GAD65 antibodies. Patients who tested positive for anti-GAD65 anti-

bodies in our study failed case ascertainment in 92% of cases, which is partly due to diagnostic criteria for AE being poorly adapted to suit GAD65-related autoimmune manifestations, although mostly due to inclusion of nonneurological cases. When using a cut-off of >2000 IU/ml on serum samples analyzed by ELISA, inclusion of nonneurological cases is inevitable [25, 26]. Some of the laboratories in Sweden did not automatically quantify GAD65-positive serum samples if the results were higher than 2000 IU/ml making it impossible for us to choose a higher cut-off. The high number of false-positive tests also explains part of the unusual sex distribution in some antibody subgroups in our study. For example, 39% of patients with anti-Yo antibodies in all of Sweden were male (Figure 2). However, during case ascertainment in Healthcare region Mid Sweden, only females met diagnostic criteria for definite PNS, and the males with anti-Yo antibodies had all been tested at the laboratory using only immunoblot. This underscores the importance of critical evaluation of test results in a real-world setting where commercial test kits are common, as both onconeural and anti-GAD65 antibodies are frequently part of test panels for AE and PNS.

Our study has several limitations that probably lead to an underestimation of the incidence of AE and PNS. We could only include 75% of the potential cases in the case ascertainment part of the study, mainly due to a lack of consent from patients. Assuming the ratio of failed case ascertainment would be the same in the excluded potential cases, the incidence rate of AE and PNS would be 4.0 per million person-years (compared with 3.0 per million person-years in our study). Further, we cannot exclude the possibility that some patients belonging to Healthcare region Mid Sweden received treatment elsewhere, thus being excluded from this study, although we consider it unlikely as the vast majority of referrals are made inside the same healthcare region in Sweden. This study only estimated the incidence of antibody-positive AE and PNS, excluding all potential antibody-negative cases. Case ascertainment relied on retrospective review of medical records, making it vulnerable to missing or inaccurate information. Extrapolation from regional to national incidence rates may be unreliable, mainly due to differences in the population of different regions. However, Sweden is a relatively homogenous country; 80% of the population are ethnic Swedes, immigrants are evenly distributed over the country and mostly from Syria, Iraq, Finland, and Poland (in that order). Furthermore, the healthcare system is organized in the same way in all regions, and there is reasonable consensus among neurologists on how to manage patients. Therefore, we chose to present incidence rates from Healthcare region Mid Sweden as our main finding, but due to the above mentioned reasons, we also presented extrapolated national incidence rates. Despite these limitations, we do believe that this study contributes important information about the epidemiology of AE and PNS in a Swedish population that can provide a foundation for healthcare planning.

In conclusion, our study adds to the increasing epidemiological data about AE and PNS by confirming that these conditions are at least equally rare as some types of

infectious encephalitides (2.4 cases of AE per million person-years in our study compared to 2.2 cases per million person-years of HSV-1 encephalitis in Sweden [27]), thus warranting the same level of awareness among physicians. Our study, designed to use data from routine healthcare, highlights some of the challenges physicians face when it comes to interpreting test results. In the future, not only heightened awareness but also increased guidance about how to diagnose these conditions and the use of neuronal antibody testing is called for.

Appendix

A. Neuronal antibodies were analyzed at five different laboratories in Sweden

A.1. Wieslab Diagnostic Services, Malmö. Time period active: 2015-2019

Yo, Hu, Ri, Ma, CV2/CRMP5, and Amphiphysin: immunoblot and indirect immunofluorescence (IIF). For IIF test, it is applied a combination of fixed tissues from the nerve, cerebellar, intestinal tissue, and pancreas originating from monkeys.

SOX1, Zic4, and Tr: immunoblot

GlyR, ANNA-3, ITPR1, HOMER3, CARPVIII, IgLON5, PCA-2, MGlur5, and MGlur1: indirect immunofluorescence (IIF)

NMDAR, AMPA 1/2, LGI1, CASPR2, DPPX, GABA B, and VGKC: indirect immunofluorescence using transfected cells (Euroimmun Lübeck).

GAD65: serum samples: ELISA, immunoblot, and indirect immunofluorescence (IIF). CSF samples: immunoblot and indirect immunofluorescence (IIF)

A.2. Uppsala University Hospital Department of Clinical Immunology and Transfusion Medicine. Time period active: 2015-2019

NMDAR, AMPA 1, AMPA 2, LGI1, CASPR2, and GABA B: 2015 until November 2016, a combination of antibody screening using neuronal tissues (hippocampus/cerebellum) and indirect immunofluorescence using transfected cells. After November 2016, only indirect immunofluorescence using transfected cells (Euroimmun Lübeck).

Hu, Ri, Yo, Ma2, CV2, Amphiphysin, Recoverin, SOX1, Titin, Zic4, GAD65, and Tr: 2015 until November 2016, a combination of antibody screening using neuronal tissues (hippocampus/cerebellum) and immunoblot. ELISA for quantification of GAD65 antibodies. After November 2016, only immunoblot.

A.3. Skåne University Hospital Department of Clinical Immunology and Transfusion Medicine. Time period active: 2015-2019

AMPA 1/2, CASPR2, DPPX, GABA B, LGI1, and NMDAR: indirect immunofluorescence using transfected cells (Euroimmun Lübeck).

Amphiphysin, CV2, Hu, Ma2, Ri, Yo, Tr, and PCA-2: immunoblot and indirect immunofluorescence (IIF). Patient sera are analyzed on neuronal tissues.

A.4. Sahlgrenska University Hospital Department of Clinical Immunology and Transfusion Medicine. Time period active: 2015-2019

AMPA 1/2, CASPR2, DPPX, GABA B, LGI1, and NMDAR: indirect immunofluorescence using transfected cells (Euroimmun Lübeck). DPPX added 2018-2019.

Hu, Ri, Yo, and Tr: immunoblot and indirect immunofluorescence (IIF)

GAD: ELISA

Ma2, CV2, SOX1, and amphiphysin: immunoblot

PCA-2: IIF

A.5. Karolinska University Laboratory Department of Clinical Immunology and Transfusion Medicine. Time period active: 2015-2019

AMPA 1/2, CASPR2, DPPX, GABA B, LGI1, and NMDAR: screening with immunofluorescence on neuronal tissues and pancreas together with indirect immunofluorescence using transfected cells (Euroimmun Lübeck).

Hu, Yo, Ri, Ma2/Ta, CV2, Amphiphysin, SOX1, Zic4, GAD65, and Tr: screening with immunofluorescence on neuronal tissues and pancreas together with immunoblot.

Quantification of GAD65 with ELISA.

Data Availability

The datasets analyzed during the current study are not publicly available due to the GDPR legislation, but are available from the corresponding author upon reasonable request.

Ethical Approval

The study was approved by the Swedish Ethical Review Authority (Dnr: 2019-03068). This study was registered at Clinicaltrials.gov (Identifier: NCT04708626).

Consent

Signed informed consent was required for inclusion in parts of the study, and the corresponding author has all the consent forms on file.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Anna Rostedt Punga and Joachim Burman shared last authorship.

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References

- [1] F. Graus, M. J. Titulaer, R. Balu et al., "A clinical approach to diagnosis of autoimmune encephalitis," *The Lancet Neurology*, vol. 15, no. 4, pp. 391–404, 2016.
- [2] F. Graus, A. Vogrig, S. Muñoz-Castrillo et al., "Updated diagnostic criteria for paraneoplastic neurologic syndromes," *Neurology - Neuroimmunology Neuroinflammation*, vol. 8, no. 4, article e1014, 2021.
- [3] D. Dubey, S. J. Pittock, C. R. Kelly et al., "Autoimmune encephalitis epidemiology and a comparison to infectious encephalitis," *Annals of Neurology*, vol. 83, no. 1, pp. 166–177, 2018.
- [4] J. Hébert, B. Riche, A. Vogrig et al., "Epidemiology of paraneoplastic neurologic syndromes and autoimmune encephalitides in France," *Neurology - Neuroimmunology Neuroinflammation*, vol. 7, no. 6, article e883, 2020.
- [5] M. S. Nissen, M. S. Ørvik, A. C. Nilsson, M. Ryding, M. Lydolph, and M. Blaabjerg, "NMDA-receptor encephalitis in Denmark from 2009 to 2019: a national cohort study," *Journal of Neurology*, vol. 269, no. 3, pp. 1618–1630, 2021.
- [6] Y. Gu, M. Zhong, L. He et al., "Epidemiology of antibody-positive autoimmune encephalitis in Southwest China: a multicenter study," *Frontiers in Immunology*, vol. 10, p. 2611, 2019.
- [7] A. van Sonderen, R. D. Thijs, E. C. Coenders et al., "Anti-LGI1 encephalitis," *Neurology*, vol. 87, no. 14, pp. 1449–1456, 2016.
- [8] L. Zuliani, S. Marangoni, P. De Gaspari et al., "Epidemiology of neuronal surface antibody-mediated autoimmune encephalitis and antibody-based diagnostics," *Journal of Neuroimmunology*, vol. 357, p. 577598, 2021.
- [9] A. Vogrig, G. L. Gigli, S. Segatti et al., "Epidemiology of paraneoplastic neurological syndromes: a population-based study," *Journal of Neurology*, vol. 267, no. 1, pp. 26–35, 2020.
- [10] M. Nosadini, M. Eyre, E. Molteni et al., "Use and safety of immunotherapeutic management of N-methyl-d-aspartate receptor antibody encephalitis: a meta-analysis," *JAMA Neurology*, vol. 78, no. 11, pp. 1333–1344, 2021.
- [11] C. Finke, H. Prüss, J. Heine et al., "Evaluation of cognitive deficits and structural hippocampal damage in encephalitis with leucine-rich, glioma-inactivated 1 antibodies," *JAMA Neurology*, vol. 74, no. 1, pp. 50–59, 2017.
- [12] S. Binks, C. Uy, J. Honnorat, and S. R. Irani, "Paraneoplastic neurological syndromes: a practical approach to diagnosis and management," *Practical Neurology*, vol. 22, no. 1, pp. 19–31, 2022.
- [13] R. Ruiz-García, E. Martínez-Hernández, A. Saiz, J. Dalmau, and F. Graus, "The diagnostic value of onconeural antibodies depends on how they are tested," *Frontiers in Immunology*, vol. 11, p. 1482, 2020.
- [14] B. Déchelotte, S. Muñoz-Castrillo, B. Joubert et al., "Diagnostic yield of commercial immunodots to diagnose paraneoplastic neurologic syndromes," *Neurology - Neuroimmunology Neuroinflammation*, vol. 7, no. 3, article e701, 2020.
- [15] A. Budhram, M. W. Nicolle, and L. Yang, "The positive predictive value of onconeural antibody testing: a retrospective review," *The Canadian Journal of Neurological Sciences*, vol. 45, no. 5, pp. 577–579, 2018.
- [16] L. McCracken, J. Zhang, M. Greene et al., "Improving the antibody-based evaluation of autoimmune encephalitis," *Neurology - Neuroimmunology Neuroinflammation*, vol. 4, no. 6, article e404, 2017.
- [17] M. Pace, G. Lanzieri, M. Glickman, and T. Zupanič, *Revision of the European Standard Population: Report of Eurostat's Task Force*, Publications Office of the European Union, 2013.
- [18] M. J. Titulaer, L. McCracken, I. Gabilondo et al., "Treatment and prognostic factors for long-term outcome in patients with anti-NMDA receptor encephalitis: an observational cohort study," *The Lancet Neurology*, vol. 12, no. 2, pp. 157–165, 2013.
- [19] M. S. Boesen, A. P. Born, M. C. Lydolph, M. Blaabjerg, and M. L. Borresen, "Pediatric autoimmune encephalitis in Denmark during 2011–17: a nationwide multicenter population-based cohort study," *European Journal of Paediatric Neurology*, vol. 23, no. 4, pp. 639–652, 2019.
- [20] F. Graus, J. Y. Delattre, J. C. Antoine et al., "Recommended diagnostic criteria for paraneoplastic neurological syndromes," *Journal of Neurology, Neurosurgery, and Psychiatry*, vol. 75, no. 8, pp. 1135–1140, 2004.
- [21] S. Shah, E. P. Flanagan, P. Paul et al., "Population-based epidemiology study of paraneoplastic neurologic syndromes," *Neurology - Neuroimmunology Neuroinflammation*, vol. 9, no. 2, article e1124, 2022.
- [22] "ECIS - European Cancer Information System," January 2022, <https://ecis.jrc.ec.europa.eu>.
- [23] C. G. Bien, C. I. Bien, M. Dogan Onugoren et al., "Routine diagnostics for neural antibodies, clinical correlates, treatment and functional outcome," *Journal of Neurology*, vol. 267, no. 7, pp. 2101–2114, 2020.
- [24] D. Dubey, N. Kothapalli, A. McKeon et al., "Predictors of neural-specific autoantibodies and immunotherapy response in patients with cognitive dysfunction," *Journal of Neuroimmunology*, vol. 323, pp. 62–72, 2018.
- [25] A. Muñoz-Lopetegui, M. de Bruijn, S. Boukhriji et al., "Neurologic syndromes related to anti-GAD65," *Neurology - Neuroimmunology Neuroinflammation*, vol. 7, no. 3, article e696, 2020.
- [26] A. Saiz, Y. Blanco, L. Sabater et al., "Spectrum of neurological syndromes associated with glutamic acid decarboxylase antibodies: diagnostic clues for this association," *Brain*, vol. 131, no. 10, pp. 2553–2563, 2008.
- [27] A. Hjalmarsson, P. Blomqvist, and B. Sköldenberg, "Herpes simplex encephalitis in Sweden, 1990–2001: incidence, morbidity, and mortality," *Clinical Infectious Diseases*, vol. 45, no. 7, pp. 875–880, 2007.