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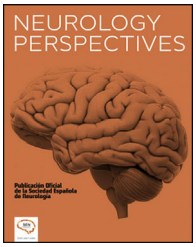

ORIGINAL ARTICLE

Autoimmune encephalitis and related disorders: A retrospective study of 43 cases in a tertiary hospital

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KEYWORDS
Autoimmune encephalitis and related disorders;
Central nervous system;
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Abstract
Background: Despite progresses in autoimmune encephalitis and related disorders (AERD), they remain a major challenge in daily clinical practice.
Objectives: To describe a tertiary hospital's longitudinal experience in AERD between 2005 and 2020.
Methods: Single-centre retrospective analysis of 43 patients.
Results: 43 patients were included, 55.8% with positive antibodies (10 antiGAD, 4 antiNMDAR, 2 antiGABABR, 2 antiLGI1, 2 antiCASPR2, 1 antiIgLON5, 1 antiHu, 1 antiYo, 1 antiCV2 and 1 antiMa2, detecting coexisting antibodies in one patient: antiNMDAR+antiGABABR); 28% with negative antibodies; and 16.2% with steroid responsive encephalitis associated to antithyroid antibodies (SREAT). The median age was 62 years-old [14–88]. Females (62.8%) outnumbered males (37.2%). Limbic encephalitis was the most common clinical syndrome (60.5%), followed by SREAT (16.2%), autoimmune cerebellitis (9.3%), stiff-person syndrome (7%), antiNMDAR-encephalitis (5%) and antiIgLON5 encephalopathy (2%). CSF showed pleocytosis and/or hyperproteinorrhachia in 54.2%. MRI was unremarkable in 60%. Brain SPECT/PET showed hyperperfusion/hypermetabolism of limbic areas in 60% of patients to whom it was performed. Antibody-positivity was significantly associated with satisfaction of diagnostic criteria at high levels of certainty ($p < 0.001$). Treatment escalation was more frequent in antibody-positive patients. The creation of a Neuroimmunology Unit improved diagnostic and treatment approaches.

Abbreviations: AAD, Antineuronal autoimmune disorders; CNS, Central nervous system; CSF, Cerebrospinal fluid; GAD, Glutamic acid decarboxylase; NMDA, N-methyl-D-aspartate; GABA, Gamma-aminobutyric acid; LGI1, Leucine Rich Glioma Inactivated 1; CASPR2, Contactin-associated protein-like 2; SREAT, Steroid responsive encephalitis associated to antithyroid antibodies; SOX1, SRY-Box transcription factor 1; PNMA2, Paraneoplastic antigen Ma2; DNER, Delta/Notch-like epidermal growth factor-related receptor; CBA, Cell based assay; HEK, Human epithelial kidney; DPPX, Dipeptidyl-Peptidase–Like Protein-6; TPO, Thyroid peroxidase; MRI, Magnetic resonance imaging; EEG, Electroencephalogram; SPECT, Single photon emission computed tomography; PET, Positron emission tomography; 18F-FDG, Fluodeoxyglucose; OCB, Oligoclonal bands; T2/FLAIR, T2 weighted/fluid attenuation inversion recovery; SPS, Stiff person syndrome; MTP, Methylprednisolone; IVIG, Intravenous immunoglobulins; ICU, Intensive care unit

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Conclusions: AERD's diagnosis and treatment have significantly improved over the last years. However, several limitations remain, particularly concerning antibody-negative AERD. The proposed diagnostic criteria might be still too reliant on antibody-positivity. Antibody-status seems to condition treatment escalation. The creation of a Neuroimmunology Unit optimized AERD's management in clinical practice.
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PALABRAS CLAVE
Antineuronal autoimmune disorders;
Autoimmune encephalitis;
Antineuronal antibodies;
SREAT;
Seronegative autoimmune encephalitis

Encefalitis autoinmunes y trastornos relacionados: estudio retrospectivo de 43 casos en un hospital terciario

Resumen
Objetivos: Describir la experiencia longitudinal de un hospital terciario en encefalitis autoinmunes y trastornos relacionados entre 2005 y 2020.
Métodos: Análisis retrospectivo unicéntrico de 43 pacientes.
Resultados: Se incluyen 43 pacientes, 55.8% con anticuerpos positivos (10 antiGAD, 4 antiNMDAR, 2 antiGABABR, 2 antiLGI1, 2 antiCASPR2, 1 antiIgLON5, 1 antiHu, 1 antiYo, 1 antiCV2 y 1 antiMa2, detectándose anticuerpos coexistentes en 1 paciente: antiNMDAR + antiGABABR); 28% con anticuerpos negativos; 16.2% con encefalopatía con respuesta a esteroides y anticuerpos antitiroideos (SREAT). Edad media = 62 años [14–88], predominio femenino (62.8%/37.2%). La encefalitis límbica fue el síndrome más frecuente (60.5%), seguida por SREAT (16.2%), cerebelitis autoinmune (9.3%), síndrome de la persona rígida (7%), encefalitis antiNMDAR (5%) y encefalopatía antiIgLON5 (2%). Un 54.2% presentó plecotosis/hiperproteinorraquia en LCR. La RM fue normal en un 60%. El SPECT/PET demostró hiperperfusión/hipermetabolismo límbico en un 60% de los casos a los que se realizó. La positividad a anticuerpos se asoció con la satisfacción de criterios diagnósticos de alta certeza ($p < 0.001$). En pacientes con anticuerpos positivos fue más frecuente la escalada terapéutica. La Unidad de Neuroinmunología optimizó el abordaje diagnóstico-terapéutico.
Conclusión: El diagnóstico y tratamiento de las encefalitis autoinmunes y trastornos relacionados se ha desarrollado significativamente en los últimos años. Sin embargo, persisten numerosas limitaciones, particularmente afectando al grupo con anticuerpos negativos. Los criterios diagnósticos propuestos podrían ser aún demasiado dependientes de la presencia de anticuerpos, la cual parece condicionar también el tratamiento. La creación de Unidades de Neuroinmunología puede optimizar el manejo de estas enfermedades en la práctica clínica.
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Introduction

Autoimmune encephalitis and related disorder (AERD) comprehend a group of diseases mediated by an autoimmune response targeting neurons.[1] Antibodies against neuronal cell-surface, synaptic and intracellular proteins are the hallmark of these disorders.[1,2] However, the presence of antineuronal antibodies cannot always be demonstrated,[1] and their absence does not exclude an autoimmune aetiology,[3] being cell-mediated immune response also involved in AERD.[2,4] Progresses in the understanding of AERD have been dramatic over the last 15 years. However, in daily clinical practice many still unanswered questions emerge regarding their diagnosis, treatment and prognosis. Hereby, the aim of this study is to describe the longitudinal experience of the Basque Country's tertiary referral hospital in AERD over the last 16 years. The primary objective of the study is to perform a descriptive analysis of diagnostic and therapeutic processes in a daily clinical practice setting. Secondary objectives include considering the correlation between our diagnosis and the proposed diagnosis criteria and analysing differences between subgroups of patients, classifying them according to antibody-status, antibody-type and clinical syndrome. Lastly, we aim to consider the impact of the creation of a Neuroimmunology Unit in our centre.

Material and methods

We conducted a single-centre retrospective analysis of the 43 patients with AERD that were diagnosed in Cruces University Hospital between 2005 and 2020.

Patients: Inclusion and exclusion criteria

Patients affected by the following conditions were eligible for the study: antibody-mediated autoimmune encephalitis, seronegative autoimmune encephalitis, autoimmune cerebellitis, stiff person syndrome (SPS), progressive encephalomyelitis with rigidity and myoclonus (PERM) and steroid-responsive

encephalopathy associated to autoimmune thyroiditis (SREAT) or Hashimoto's encephalopathy. Regarding SREAT, it is still controversial whether its pathogenesis is autoimmune or not.[5,6] However, it might clinically resemble autoimmune encephalitis.[3] Since this study is focused on daily clinical practice, SREAT was included. The diagnosis of autoimmune encephalitis was made based on clinical syndrome (subacute onset of working memory deficits, altered level of consciousness, psychiatric symptoms, seizures and/or focal deficits), supported by the presence of antineuronal antibodies in the case of antibody-mediated autoimmune encephalitis; or by either an altered and congruent brain magnetic resonance imaging (MRI) (hyperintense signal on T2-weighted/fluid-attenuated inversion recovery (T2/FLAIR) sequences of one or both medial temporal lobes or in multifocal areas compatible with inflammation) and/or CSF (pleocytosis, hyperproteinorrachya, positive oligoclonal bands (OCB)), and/or cancer coexistence, suggesting a paraneoplastic etiology; in the case of seronegative autoimmune encephalitis. The diagnosis of autoimmune cerebellitis was made based on clinical syndrome (subacute onset of limb and/or troncular ataxia, dyshartic speech and gait disturbances) supported by the presence of either antineuronal antibodies, an altered and congruent MRI (early hyperintense signal on T2/FLAIR sequences of the vermis and cerebellar folia compatible with inflammation or delayed cerebellar atrophy), CSF (pleocytosis, hyperproteinorrachya, positive OCB), and/or cancer coexistence, suggesting a paraneoplastic etiology. SPS and PERM were diagnosis based on the presence of stiffness, rigidity and spasms and either an electromyography showing continuous motor unit activity that persists despite voluntary attempts at muscle relaxation or positive antiGAD or anti-glycine receptor autoantibodies. The diagnosis of SREAT was made based on the clinical syndrome (subacute onset of seizures, myoclonus, memory deficits, confusion, hallucinations and/or stroke-like episodes) and the presence of serum antithyroid peroxidase antibodies. A reasonable exclusion of alternative diagnosis was required for all disorders. In the case of SREAT, negative antineuronal antibodies and euthyroidism/subclinical thyroid disease were needed. The exclusion criteria were defined as follows: autoimmune disorders targeting glial cells or myelin; autoimmune diseases of the peripheral nervous system; coexisting systemic autoimmune disease potentially affecting the central nervous system; and the finding of antineuronal antibodies without clinical correlation. A comprehensive search of cases with above-mentioned disorders between 2005 and 2020 was conducted in our centre. 43 patients entered the study. 24 had positive antineuronal antibodies, 12 had negative antibody-tests and 7 had SREAT.

Antibody detection methods

The immunology laboratory of Cruces University Hospital analysed serum and CSF samples for intracellular antibodies from 2008 to 2020. The laboratory used indirect immunoblot assays (commercial kit, EUROIMMUN, EUROLINE) for detection of antiHu, antiYo, antiRi, antiCV2, antiPNMA2(Ma2/Ta), antiamphiphysin, antiRecoverin, antiSOX1, antiZic4, antiGAD and antiTr(DNER). Positive results were confirmed using indirect immunofluorescence assays on primate's nerve, gut and cerebellar tissue (EUROIMMUN). In the case of antibodies against neuronal cell-surface/synaptic proteins, the laboratory performed a cell-based assay (CBA) on human epithelial kidney (HEK) transfected cells (commercial kit, EUROIMMUN) analysing antiNMDAR, antiGABABR, antiAMPA, antiLGI1, antiCASPR2 and antiDPPX autoantibodies. CBA became available in our centre in 2017. Before 2008 in the case of intracellular antibodies and before 2017 in the case of antineuronal cell-surface/synaptic antibodies, serum and CSF samples were sent to the immunology laboratory of Clinic Hospital (Barcelona), where the following antibodies were analysed: antiHu, antiYo, antiRi, antiCV2, antiMa2, antiamphiphysin, antiRecoverin, antiSOX1, antiGAD and antiTr (DNER), antiNMDAR, antiGABAAR, antiGABABR, antiAMPAR, antiLGI1, antiCASPR2, antiDPPX, antimGluR1, antimGluR5, antiNeurexine and antiIgLON5. The laboratory performed a first screening using indirect immunochemistry on rat's brain and cerebellar tissue. In case of a positive result, immunoblot assays were conducted in the case of intracellular antibodies and CBA in the case of antineuronal cell-surface/synaptic antibodies (in-house techniques). After 2017, selected patients' samples were analysed in both Cruces University Hospital and Clinic Hospital, including samples of three patients with negative antibody results and of two patients with positive antibodies (in order to confirm their positivity). If a disorder mediated by any antibody that was not available in Cruces University Hospital's lab was suspected, samples were sent directly to Clinic Hospital. Antithyroperoxidase antibodies were analysed in Cruces University Hospital using chemoluminescence competitive immunoassay (AtellicaTM, Siemens).

Data collection and statistical analysis

The following data were recorded: gender; age; clinical syndrome; antibody determination on serum and CSF; MRI; lumbar puncture, OCB; electroencephalogram; brain single photon emission computerized tomography (SPECT) and/or brain fluodeoxyglucose positron emission tomography (18F-FDG PET); underlying tumour; tumour markers; acute phase treatment; outcomes and mortality. The following tumour markers were assessed: carcinoembryonic antigen, cancer antigens 15.3, 19.9 and 125 and alpha-fetoprotein, tested using direct chemo-luminescence immunoassay, AtellicaIM1600, Siemens; serum squamous cell carcinoma, tested using chemiluminescent microparticle immunoassay, Architect i1000SR; and neuron-specific enolase and CYFRA 21.1, tested using electrochemiluminescence immunoassay, Elecsys Cobas e801, Roche. Eligible first line therapies were intravenous pulses of methylprednisolone (1 g/day during 4–5 days), intravenous immunoglobulins (IVIG) (0.4 g/kg/day during 5 days) and plasmapheresis. As second-line treatment rituximab was used. Both the combination of first-line therapies between each other or with a second-line treatment were considered treatment escalation. Outcomes were assessed based on clinical aspects. Good outcomes were considered in case of clinical improvement (complete or partial resolution of symptoms). Poor outcomes were considered in case of no clinical improvement, clinical impairment and/or death. Correlation between each entity and proposed diagnosis criteria was assessed: in the case of autoimmune encephalitis, limbic encephalitis, seronegative autoimmune encephalitis and SREAT, correlation to diagnosis criteria that were proposed in 2016,[3] in the case of antiNMDAR-

encephalitis, correlation to diagnosis criteria proposed in 2019,[7] in the case of paraneoplastic syndromes, correlation to diagnosis criteria proposed in 2008 [8] and in SPS, correlation to diagnosis criteria proposed in 2020 [9] (Appendix). Differences between subgroups of patients were analysed, according to antibody-status (positive, negative), antibody-type (anti-neuronal cell-surface/synaptic protein, onconeuronal, antiGAD) and clinical syndrome (limbic encephalitis, antiNMDAR-encephalitis, autoimmune cerebellitis, SPS, SREAT). The chi-squared test or Fisher's exact test was used to compare differences among the subsets of qualitative variables. The independent-sample t-test and analysis of variance were used to compare differences in continuous quantitative variables. For statistical analysis SPSS 24.0 software was used, with a "p" value of <0.05 indicating a significant difference.

Results

A total of 43 patients with AERD were included: 24 (55.8%) antibody-positive, 12 (28%) antibody-negative and 7 (16.2%) SREAT. Among the 24 antibody-positive patients, 10 (41.6%) had anti-neuronal cell-surface/synaptic protein antibodies. 16 (66.6%) had intracellular antibodies. Antineuronal antibodies were, in order of frequency: 10 antiGAD, 10 antineuronal cell-surface/synaptic (4 antiNMDAR, 2 antiGABABR, 2 antiLGI1, 2 antiCASPR2, 1 antiIgLON5) and 4 onconeuronal (1 antiHu, 1 antiYo, 1 antiCV2 and 1 antiMa2). In one patient, coexisting antibodies were detected: antiNMDAR+antiGABABR. Being all cases diagnosed between 2005 and 2020, the median time of follow-up was 2 years and 9 months [<1 month–15 years]. An increasing raise in the incidence of recognized and diagnosed cases of AERD was observed over time, reaching the most substantial increment over the last four years (Fig. 1).

The median age was 62 years-old [14–88], with no significant differences depending on antibody-status. However, age-differences depending on antibody-subtype and clinical syndrome were found (Fig. 2). Patients with anti-neuronal cell-surface/synaptic antibodies tended to be younger (median of 56); while patients with onconeuronal antibodies tended to be older (median of 65). Patients with

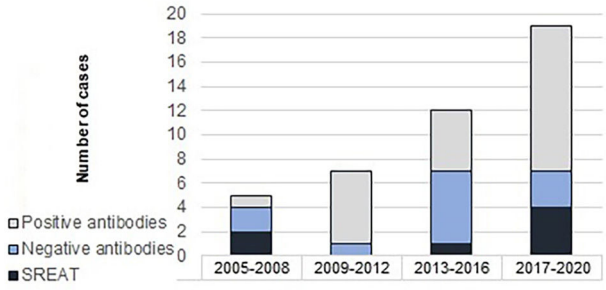

Fig. 1 Case incidence distribution per four-year period. All the cases were diagnosed between 2005 and 2020. However the incidence of autoimmune encephalitis and related disorder has been increasingly raising until reaching the most substantial increment during the last four years. The incidence between 2017 and 2020 (19 cases) almost quadruples the incidence between 2005 and 2008 (5 cases), being 12 times higher in the case of patients with positive antibodies (12 versus 1).

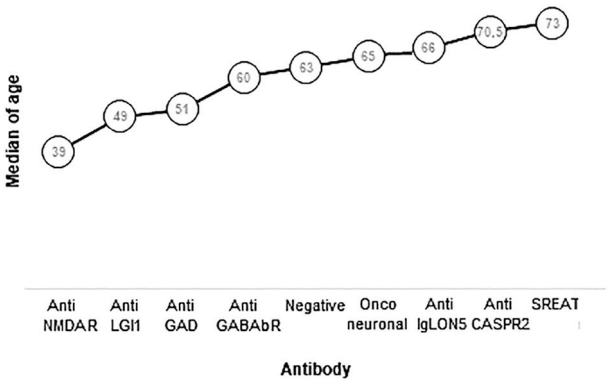

Fig. 2 Age distribution per antibody type. Age-differences depending on antibody-subtype were found. Patients with antiNMDAR antibodies were the youngest (median age of 39 years-old), followed by patients with antiLGI1 antibodies (median of 49) and antiGAD (median of 51). Among patients with anti-neuronal cell-surface antibodies, patients with antiCASPR2 antibodies were the oldest (median of 70.5). Patients with SREAT were older than all the other subgroups (median age of 73). *CASPR2: Contactin-associated protein-like 2, GABAbR: gamma-aminobutyric acid b receptor, GAD: glutamic acid decarboxylase, IgLON5: immunoglobulin-like cell adhesion molecule 5, LGI1: Leucine Rich Glioma Inactivated 1, NMDAR: N-methyl-D-aspartate receptor, SREAT: Steroid responsive encephalopathy with antithyroid antibodies.

antiNMDAR-encephalitis (median of 24.5) were the youngest. Patients with SREAT (median of 73) were the oldest. Regarding sex, 27 patients (62.8%) were females and 16 (37.2%) were males. Differences on sex distribution depending on antibody-subtype were found. All patients with antiNMDAR-encephalitis and with antiLGI1 antibodies were female (100%). Females outnumbered males also in the case of SREAT (85.7%), antiGAD (80%) and seronegative autoimmune encephalitis (58%). In contrast, onconeuronal (80%), antiGABABR and antiCASPR2 (100%) antibodies were more frequent in men.

Regarding clinical syndromes, limbic encephalitis was the most common (60.5%), being antibody-mediated in 54% and seronegative in 46%; followed by SREAT (16.2%), autoimmune cerebellitis (9.3%), SPS (7%) and antiNMDAR-encephalitis (5%). AntiIgLON5 encephalopathy affected one patient.

Regarding antibody detection, anti-neuronal cell-surface/synaptic and intracellular antibodies were assessed in all patients in both blood and CSF samples (except one patient, due to lumbar puncture refusal). Among the 24 patients with positive antibodies, 95.8% had antibodies in serum, 87.5% in CSF and 83.3% in both serum and CSF. In three patients, antibodies were positive only in serum (both antiLGI1, one antiGABABR). One patient presented positive antibodies only in CSF (coexisting antiNMDAR+antiGABABR). CSF showed pleocytosis [6–140 cells/mm^3] and/or hyperproteinorrhachia [45–650 mg/dl] in 54.2% of patients. There was not significant association between abnormal CSF and antibody-positivity. Moreover, antiLGI1 and antiGAD patients tended to have normal CSF. OCB were assessed in 15 patients, resulting positive in 4.

Brain MRI was unremarkable in 60% of patients. Only six patients showed the characteristic hyperintense signal of

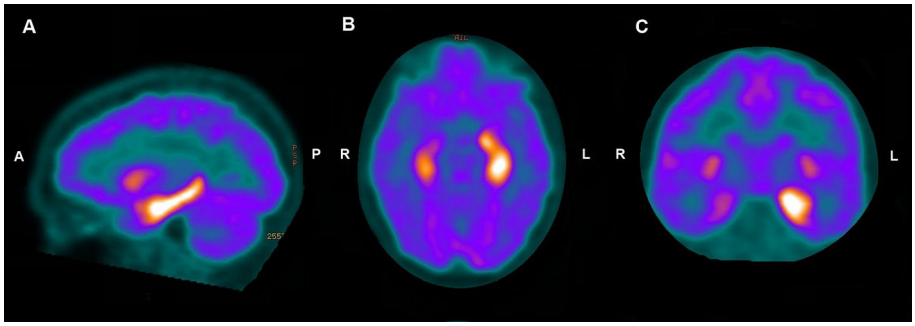

Fig. 3 Brain 18F-FDG PET. Brain 18F-FDG PET, sagittal slide (A), axial slide (B) and coronal slides (C). A: Anterior. P: Posterior. R: Right. L: Left. Left predominant but bilateral medial temporal lobes and limbic system hypermetabolism in a patient with antiGAD67 antibodies is shown. Left hippocampus, fornix and cingulate cortex hypermetabolism can be seen in sagittal slides (A). Left predominant but bilateral hippocampus (B) hypermetabolism can be seen in axial slides. Left amygdala's hypermetabolism is evident in coronal slides (C).

both medial temporal lobes on T2/FLAIR sequences. This finding was more frequent in antibody-negative patients (33.3%) than in antibody-positives (8.3%). There was no significant association between altered MRI and antibody-positivity. Electroencephalography was abnormal in 68%. Epileptic activity was registered in 44% (20% with status epilepticus) and slow brain activity in 24%. Functional nuclear medicine studies of the brain were performed in 15 patients, considering both brain SPECT (evaluating blood flow) and brain 18F-FDG PET (evaluating glucose metabolism). They showed hyperperfusion/hypermetabolism of limbic areas (Fig. 3) in 60% and hypoperfusion/hypometabolism of frontal and/or temporal lobes in 20%. Hyperperfusion/hypermetabolism was evident in all antiNMDAR patients who underwent a brain SPECT/PET, despite normal MRI results. In patients with abnormal MRI, an overlap between T2/FLAIR hyperintensity and hyperperfusion/hypermetabolism was evident (Fig. 4).

Screening for underlying malignancy detected tumours in 11 patients (25.6%). The most common tumour was lung cancer (55%), followed by teratoma (27%) and ovarian adenocarcinoma (18%). Paraneoplastic syndromes were limbic encephalitis, antiNMDAR-encephalitis and autoimmune cerebellitis. They were associated to antiHu, antiCV2, antiYo, antiCASPR2, antiGABABR and antiNMDAR antibodies. Four patients were seronegative. Onconeuronal antibodies were statistically associated with cancer ($p < 0.009$). Tumour markers were assessed in 27 patients (not assessed in antiGAD, in one antiNMDAR-encephalitis, in two seronegative patients and in three SREAT). 10 of them were finally diagnosed with neoplasm, finding high levels of tumour markers in 6 and normal values in 4. Additionally, two patients without detectable tumour showed raised values of tumour markers. Status epilepticus, altered MRI and/or CSF were more common among patients with tumours.

Regarding correlation to diagnostic criteria, it was high for patients with autoimmune encephalitis and 'possible autoimmune encephalitis' criteria (92%). Only three patients who were predominantly affected by seizures and focal deficits did not meet them. But, overall, only 48.5% of patients met criteria at higher levels of certainty. This ratio significantly increases between antibody-positive patients, of whom almost 90% satisfied the proposed criteria at high levels of certainty ('definite limbic encephalitis', 'definite antiNMDAR-encephalitis'). On the other hand, only 25% of seronegative patients fulfilled 'definite limbic encephalitis' criteria and only 33.3% fulfilled criteria for 'autoantibody-negative but probable autoimmune encephalitis'. All patients with tumour fulfilled the proposed diagnostic criteria

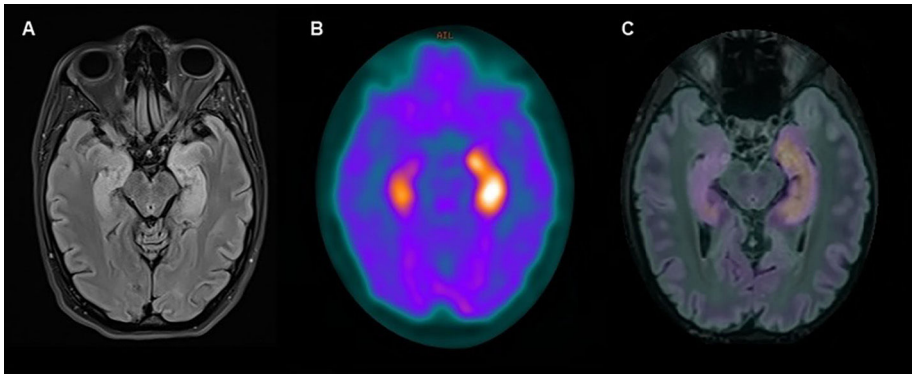

Fig. 4 Fusion of brain MRI and 18F-FDG PET. Fusion of brain MRI and 18F-FDG PET of a patient with antiGAD67 autoimmune encephalitis is shown, axial slides (A) Brain MRI (axial FLAIR sequence): left predominant but bilateral medial temporal lobes hyperintensity can be seen. (B) Brain 18F-FDG PET (axial slide): left predominant but bilateral hippocampus hypermetabolism can be seen. (C) Brain MRI and 18F-FDG PET fusion shows an overlap between T2/FLAIR hyperintensity and hypermetabolism.

Table 1 Comparison of patients according to antibody-status.

Variable	Antibody-status		p value
	Antibody-positive (n = 24)	Antibody-negative (n = 12)	
Sex (females/males)	58.3%/41.7%	58%/42%	1
Age (mean)	54	51.7	0.396
Clinical sings/symptoms			
Seizures	13 (54.1%)	7 (58.3%)	1
Memory deficits	14 (58.3%)	6 (50%)	0.729
Behavioural changes	10 (41.6%)	6 (50%)	0.729
Psychotic symptoms	6 (25%)	4 (33.3%)	0.7
Dysautonomia	2 (8.3%)	0	0.543
Ataxia	7 (26.1)	2 (16.6%)	0.685
Involuntary extrapyramidal movements	7 (29.1%)	0	0.07
Altered MRI	10 (4.1%)	7 (58.3%)	0.483
CSF pleocytosis/hyperproteinorrachia	14 (60.8%)	7 (58.3%)	1
Brain 18F-FDG PET/SPECT hyperperfusion/hypermetabolism	5/9 (55.5%)	3/4 (75%)	1
Possible autoimmune encephalitis criteria	16 (88.8%)	11 (91.6%)	1
High level of certainty criteria (definite limbic encephalitis; definite antiNMDAR-encephalitis)	15 (88.2%)	3 (25%)	**0.001**
Antibody negative but probable autoimmune encephalitis criteria	–	4 (33.3%)	–
Treatment			
Monotherapy	8 (33.3%)	6 (50%)	0.458
Treatment escalation	15 (62.5%)	5 (41.6%)	0.458
Outcomes			
Good outcomes	16 (66.6%)	8 (66.6%)	1
Poor outcomes	8 (33.3%)	4 (33.3%)	1
Mortality	7 (29.1%)	4 (33.3%)	1

Statistically significant results appear in bold-type.
Abbreviations: CSF: cerebrospinal fluid, MRI: Magnetic resonance imaging, NMDAR: N-methyl-D-aspartate receptor, PET: positron emission tomography, SPECT: single photon emission computed tomography, 18F-FDG: Fluodeoxyglucose.

for 'probable paraneoplastic syndrome' and 91% for 'definite paraneoplastic syndrome'. Regarding SPS, 80% met the proposed diagnostic criteria. Regarding SREAT, all but one satisfied the criteria for 'Hashimoto's encephalopathy'. Antibody-positivity was significantly associated with the satisfaction of diagnosis criteria at high levels of certainty ($p < 0.001$) (Table 1).

Regarding acute phase treatment, monotherapy with pulses of methylprednisolone alone was used in 31% and with IVIG in 16.7%. No patient received plasmapheresis in monotherapy. Pulses of methylprednisolone plus IVIG was the most common treatment combination (26.2%). Pulses of methylprednisolone plus plasmapheresis were used in only one case. Eight patients (18.6%) received rituximab. Treatment escalation was more frequent in antibody-positive patients ($p = 0.054$). SREAT was significantly associated with the use of monotherapy ($p < 0.031$). 7 patients (16.7%) were admitted to intensive care unit (ICU), (3 antibody-negative, 2 antiNMDAR-encephalitis, 1 antiNMDAR + antiGABABR and 1 SREAT). Four cases did not receive immunosuppressive treatment, two of them because the diagnosis was achieved post-mortem.

A total or partial clinical improvement was achieved in 70% of patients. Good outcomes were more frequent in patients with antineuronal cell-surface/synaptic antibodies (90%). Patients with onconeuronal antibodies had the worst outcomes, with none of them improving after treatment. These differences depending on antibody-type were significant ($p < 0.011$) (Table 2). Mortality rate was 30%, being related to neurological deterioration in 23%. The median time to death was 4 months. Mortality was higher in patients with onconeuronal antibodies (75%) than in other antibody-positive patients (20% in patients with antineuronal surface and with antiGAD antibodies).

Discussion

The purpose of this study is to describe the longitudinal experience of a tertiary hospital in AERD over the last 16 years from the perspective of daily clinical practice.

Among the 43 patients who were included, 55.8% had positive antibody-tests. Similarly, in previously published series, approximately 50% of autoimmune encephalitis were negative to antibody-assays.[10,11] Antibody-positivity was significantly associated with satisfaction of diagnostic criteria at high levels of certainty ($p < 0.001$). Moreover, only 33% of patients with negative antibodies fulfilled criteria for 'autoantibody-negative but probable autoimmune encephalitis'. It is worth noting that, if the antineuronal antibodies had not been detected in the antibody-positive subgroup, only 32% of them would have satisfied the latest criteria. Therefore, it might be considered if current diagnosis criteria are too reliant on the presence of antibodies. Considering diagnostic criteria for

Table 2 Comparison of patients according to antibody-type.

Variable	Antibody-type			p value
	Antineuronal surface/synaptic protein antibodies (n = 10)	Onconeuronal antibodies (n = 4)	AntiGAD (n = 10)	
Sex (females/males)	50%/50%	20%/80%	80%/20%	0.168
Age (mean)	53.6	65	50.2	0.460
Clinical sings/symptoms				
Seizures	7 (70%)	1 (25%)	5 (50%)	0.311
Memory deficits	5 (50%)	2 (33.3%)	2 (50%)	0.649
Behavioural changes	6 (60%)	0	4 (40%)	0.122
Psychotic symptoms	3 (30%)	0	0	0.209
Dysautonomia	2 (20%)	0	0	0.638
Ataxia	1 (10%)	1 (25%)	3 (30%)	0.062
Involuntary extrapyramidal movements	6 (60%)	1 (75%)	0	**0.014**
Altered MRI	3 (30%)	2 (33.3%)	5 (50%)	0.649
CSF pleocytosis/hyperproteinorrachia	8 (80%)	4 (100%)	2 (20%)	**0.011**
Brain 18F-FDG PET/SPECT hyperperfusion/ hypermetabolism	3/4 (75%)	0	2/4 (50%)	0.714
Tumour	4 (40%)	3 (75%)	0	**0.014**
Cancer	2 (20%)	3 (75%)	0	**0.009**
Treatment				
Monotherapy	3 (30%)	1 (25%)	4 (40%)	1
Treatment scalation	7 (70%)	2 (50%)	6 (60%)	1
Outcomes				
Good outcomes	9 (90%)	0 (0%)	7 (70%)	**0.011**
Poor outcomes	1 (10%)	4 (100%)	3 (30%)	**0.011**
Mortality	2 (20%)	3 (75%)	2 (20%)	0.086

Statistically significant results appear in bold-type.
Abbreviations: CSF: cerebrospinal fluid, GAD: Glutamic acid decarboxylase, MRI: Magnetic resonance imaging, NMDAR: N-methyl-D-aspartate receptor, PET: positron emission tomography, SPECT: single photon emission computed tomography, 18F-FDG: Fluodeoxyglucose.

definite autoimmune limbic encephalitis and for autoantibody-negative but probable autoimmune encephalitis,[3] we found some limiting-factors for antibody-negative patients, such as the request of brain biopsy in case of normal MRI or normal CSF in 'autoantibody-negative but probable autoimmune encephalitis' criteria. The request of temporal-lobe abnormalities on MRI being bilateral in 'definite limbic encephalitis' criteria was limiting too for seronegative patients, but not for antibody-positives. However, it should be pointed out that regular OCB assessment could have facilitated diagnosis criteria fulfilment in seronegative patients. On the other hand, it could be argued that diagnosis criteria are accurate but patients were misdiagnosed with seronegative autoimmune encephalitis. As previously mentioned, they were diagnosed only after exclusion of alternative diagnosis and supported by congruent findings on complementary assays. Few studies [12–14] have previously assessed the application of diagnostic criteria for autoimmune encephalitis in clinical practice. They did also find them dependent on antibody-positivity, excepting 'possible autoimmune encephalitis' criteria.

Diagnosis of AERD becomes challenging because typical MRI is uncommon [15] and because a normal CSF does not exclude an autoimmune aetiology.[3,10] In our series, only 14% of patients showed the typical MRI. CSF protein level and cell count resulted normal in 45.8% of patients, including 33.3% of those with positive antibodies in CSF and 75% of patients with OCB. Hence, in case of clinical suspicion, antineuronal antibodies should be assessed both in serum and CSF, regardless normal MRI and/or CSF results.[3,16] Considering the diagnostic difficulties, the role of brain SPECT/PET in AERD should be highlighted, as they seem to be more sensitive than MRI.[3,17] In our series, more than one half of the patients with hyperperfusion/hypermetabolism in SPECT/PET had normal MRI. They might be particularly useful in antiNMDAR-encephalitis,[18] in which MRI is usually normal.[7,19] All patients with antiNMDAR-encephalitis who underwent a SPETC/PET showed hyperperfusion/hypermetabolism. Hyperperfusion/hypermetabolism was also detected in 75% of antibody-negative patients to whom a SPECT/PET was performed. Giving the difficulties for fulfilling diagnosis criteria in this subgroup of patients, SPECT/PET could have a significant role in the future as an alternative to MRI criterion in 'autoantibody-negative but probable autoimmune encephalitis' criteria, as PET is accepted as an alternative to MRI in 'definite limbic encephalitis' criteria.[3]

Notwithstanding, although the diagnostic approach to antibody-negative AERD should be improved, trying to identify specific antibodies is crucial in every case.[3] Interestingly, coexisting antibodies might be detected in serum and CSF, although the significance of that coexistence has not been entirely elucidated.[20–22] Antibodies' recognition supports the diagnosis and adds a prognostic value,[3] conditioned by the different response of each antibody to immunosuppressive

therapies and by their different association with cancer. However, it is worth mentioning that tumours were also diagnosed in up to 33% of our seronegative patients. Hence, screening of malignancy should be conducted also in antibody-negative patients. It is also noteworthy that, although antibody-negative patients showed better response to treatment than patients with intracellular antibodies, treatment escalation was less frequent in the antibody-negative subgroup. Moreover, although the efficacy of rituximab in autoimmune encephalitis regardless of antibody-status has been suggested,[23] the use of rituximab was almost exclusive in antibody-positive patients. Thus, antibody-status seems to condition treatment escalation. The fact that two patients did not receive any treatment because of post-mortem diagnosis also indicates the existing reluctance to treat AERD in absence of antibodies. Nevertheless, antibody-negative autoimmune encephalitis could be severe, as evidenced by the fact that almost one-half of the patients requiring ICU admission in our series were seronegative. Hence, high efficiency treatments should not be dismissed.

Since Neuroimmunology Unit creation in 2017, AERD' diagnostic and treatment approaches have been improved in our centre. On one side, early combination of pulses of methylprednisolone and IVIG or plasmapheresis has been implemented.[10,24] On diagnosis side, laboratory detection methods for anti-neuronal cell-surface/synaptic antibodies, CBA, have been introduced, leading to greater access to tests and faster accessibility of results. This fact, along with a higher diagnosis suspicion of AERD,[13] has led to an increase in the number of diagnosed cases since 2017, clustering a 50% of all our diagnosis between 2017 and 2020. Moreover, the incidence between 2017 and 2020 almost quadruples the incidence between 2005 and 2008, being 12 times higher in antibody-positive patients. Lastly, Neuroimmunology Unit provided the proper framework for patients' follow-up.

It should be noted that, despite adding CBA to our laboratory, the role of the laboratory of reference is essential, particularly in the case of antibody-negative patients, due to their greater expertise, their access to greater panels of antibodies and due to the possibility to investigate on new antibodies.[3,11]

This study has several limitations. This is a single-centre study and includes a small number of cases (n = 43). This study is retrospective, entailing potential bias related to a non-systematized data recording. We included only patients who had been diagnosed of AERD. Accordingly, it is possible that some patients for whom the diagnosis of AERD was missed were not included. The study deals with the difficulty of including patients selected from a long period of time for studying a group of disorders whose management has progressed over time. As a result, we are aware that some antibodies were not tested because specific assays were not available or because those antibodies were unknown at the time of the diagnosis. Hence, recently described antibodies might be underexpressed. However, this study does also have some strengths and these potential bias might be excusable for our aim. Being focused on daily clinical practice, this study analyses the longitudinal experience of a tertiary centre in AERD in a real clinical setting, a quality that makes it valuable for the majority of hospitals.

Conclusions

AERD are potentially treatable conditions, whose diagnosis and treatment had not been possible until their recent description a few decades ago. Our diagnostic ability has increased over the last years and will probably continue to improve, with brain SPECT/PET potentially becoming relevant diagnostic tools. However, several limitations remain in daily clinical practice, particularly concerning antibody-negative AERD. The proposed diagnostic criteria might be too reliant on antibody-positivity. Antibody-status seems to condition treatment escalation. The creation of a Neuroimmunology Unit optimized the management of AERD in our centre.

Ethical standards statement

This study has being examined and approved by the Clinical Research Ethics Committee of Cruces University Hospital. We ensure ethical standards protecting the confidentiality and anonymity of our patients. Only clinical data are presented, avoiding any patient's personal data and photography.

Assurances

All authors declare that this work has not been published before (neither in English nor in any other language) and that this work is not under consideration for publication elsewhere.

Declaration of Competing Interest

None. On behalf of all authors, the corresponding author states that there is no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neurop.2021.09.001.

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