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ORIGINAL COMMUNICATION

# Acute pediatric encephalitis: etiology, course, and outcome of a 12-year single-center immunocompetent cohort

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## Abstract

**Background** Encephalitis is an uncommon but severe disorder due to an inflammation of the brain parenchyma, usually diagnosed on clinical, laboratory, electroencephalographic, and neuroradiological features. New causes of encephalitis have been reported in recent years, so diagnostic criteria have changed over time.

We report on a single-center experience of a pediatric Hospital, the hub of its region, over 12 years (2008–2021), with the evaluation of all children managed for acute encephalitis.

**Methods** We retrospectively reviewed clinical, laboratory, neuroradiological, and EEG data from the acute phase and outcome of all immunocompetent patients diagnosed with acute encephalitis. According to the newly proposed criteria for pediatric autoimmune encephalitis, we divided patients into infectious, definite autoimmune, probable autoimmune, and possible autoimmune, and performed a comparison between the different groups.

**Results** 48 patients (26 females, mean age 4.4 years), 19 with infections, and 29 with autoimmune encephalitis, were included. Herpes simplex virus 1 encephalitis was the most frequently identified etiology followed by anti-NMDA receptor encephalitis. Movement disorders at onset and a longer hospital stay were observed more frequently in autoimmune compared to infectious encephalitis ($p\ p < 0.001$ and $p = 0.001$**,** respectively).

Among the autoimmune group, children who started immunomodulatory treatment earlier (within 7 days from onset) had more frequent complete functional recovery ($p = 0.002$).

**Conclusions** Herpes virus and anti-NMDAR encephalitis are the most frequent etiologies within our cohort. Clinical onset and course are extremely variable. Since early immunomodulatory treatment was associated with a better functional outcome, our data confirm that a timely diagnostic classification in definite, probable, or possible autoimmune encephalitis can help the clinician in a successful therapeutic approach.

**Keywords** Infectious encephalitis · Autoimmune encephalitis · Brain inflammation · Antineuronal antibodies · Early antiviral and immunomodulatory treatment

## Abbreviations

| | |
|---|---|
| MRI | Magnetic resonance imaging |
| PICU | Pediatric intensive care unit |
| IE | Infectious encephalitis |
| CSF | Cerebrospinal fluid |
| PCR | Polymerase chain reaction |
| AE | Autoimmune encephalitis |
| ADEM | Acute disseminated encephalomyelitis |
| EDSS | Expanded disability status scale |
| IVIG | Intravenous immunoglobulin |
| PLEX | Plasma exchange |
| SWI | Susceptibility-weighted images |
| FLAIR | Fluid-attenuated-inversion-recovery |
| EBV | Epstein barr virus |

## Introduction

Encephalitis is a serious condition defined as inflammation of the brain parenchyma associated with neurologic dysfunction. Although pathologic examination of brain tissue is considered the "gold standard" diagnostic test, this is

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rarely done due to potential morbidity associated with an aggressive neurosurgical procedure. In the absence of pathologic confirmation, encephalitis has been defined based on selected clinical, laboratory, electroencephalographic, and neuroimaging features [1].

Although rare, encephalitis still represents a significant cause of mortality and neurological morbidity throughout the world. The annual incidence of encephalitis, regardless of the cause, is around 5–8 cases per 100,000 people with a peak incidence in pediatrics, especially in the first year of life, mostly for infectious etiologies [2].

In the past decades, a higher incidence has been reported compared to the past, probably in relation to the increased use of immunosuppressive therapies and transplantations, as well as to the improvement of diagnostic techniques, potentiation of brain magnetic resonance imaging (MRI), and the identification of new autoantibodies related to autoimmune etiologies [2–5]. Advances in the field of autoimmune encephalitis pathogenesis led to the identification of new syndromes and biomarkers, which led to a change in the diagnostic and therapeutic approach of entities without a clear infectious etiology [4, 5].

However, despite extensive investigation looking for infectious and autoimmune markers, the cause of encephalitis remains unexplained in almost half of the cases [6].

Considering that mortality rates improve significantly with the early start of treatment [7–9], prompt recognition and differentiation among infectious and autoimmune diseases are, therefore, mandatory, as they respond to completely different therapeutic management.

We retrospectively evaluated all pediatric patients with a diagnosis of acute encephalitis managed at a tertiary pediatric hospital over the past 12 years. We compared their clinical data at onset and follow-up according to the newly proposed classification criteria for pediatric autoimmune encephalitis [5].

## Materials and methods

IRCCS Istituto Giannina Gaslini (IGG), Genoa, Italy, is a tertiary care children's hospital in northern Italy serving as local pediatric hospital for the Genoa area, it is the hub of the Region Liguria for pediatric emergencies and represents a tertiary care referral hospital for Italy and many foreign countries.

Clinical records of all subjects < 18 years admitted at IGG with a diagnosis of acute encephalitis, defined according to International Criteria [1] in the period January 1st 2008–June 31st 2021, were first anonymized and then retrospectively reviewed.

The study was reviewed and approved by the Regional Ethics Committee.

### Data collection and diagnostic criteria

Diagnoses were reviewed by a team composed of an expert in child neurology (MMM), an infectious disease expert (EC), a pediatrician (GB), and an intensive care specialist (SB) with expertise in pediatric neurology, from independent medical record review and categorized as infectious or autoimmune encephalitis. Autoimmune encephalitis were further divided according to the recent proposal on a clinical approach to this diagnosis [5].

Both electroencephalographic and neuroradiological (TC and/or MRI) data were collected and re-evaluated by an expert epileptologist (MMM) and two neuroradiologists (DT, GM), respectively.

Specifically, demographic details, family and personal antecedents, recent history of infection or booster, clinical, serological, cerebrospinal fluid (CSF), EEG, and neuroradiological data at onset, treatment, outcome, and follow-up data were collected.

Patients with congenital or acquired immunodeficiency, with a diagnosis of acute disseminated encephalomyelitis (ADEM), or with anti-MOG positivity, and patients affected by infectious encephalitis but with predisposing genetic factors (such as RANBP2) mutation were excluded, as shown in Fig. 1.

#### Infectious encephalitis

Infectious encephalitis (IE) was defined by a clinical and eventually neuroradiological diagnosis [1] combined with the identification of a viral or bacterial pathogen potentially cause of encephalitis, in blood or cerebrospinal fluid (CSF) cultures or using a polymerase chain reaction (PCR) or by a positive serology (IgM) with clinical evidence of acute infection of an organism strongly associated to encephalitis.

#### Autoimmune encephalitis

Autoimmune encephalitis (AE) was defined according to the new proposal for pediatric AE [5].

The initial criteria for Possible AE include the following: (1) evidence of acute or subacute symptom onset (2) two or more clinical evidence of neurological dysfunctions (3) exclusion of other etiologies.

Criteria for Probable AE include all the above-mentioned points (1), (2), (3) plus 1 or more features among (a) CSF inflammatory changes (b) MRI findings of encephalitis (c) brain biopsy evocative of inflammation. Criteria for Definite AE included all criteria for Probable AE (not necessary (a), (b), (c) in the case of anti-NMDARs encephalitis) with the presence in the serum and/or CSF of well-characterized

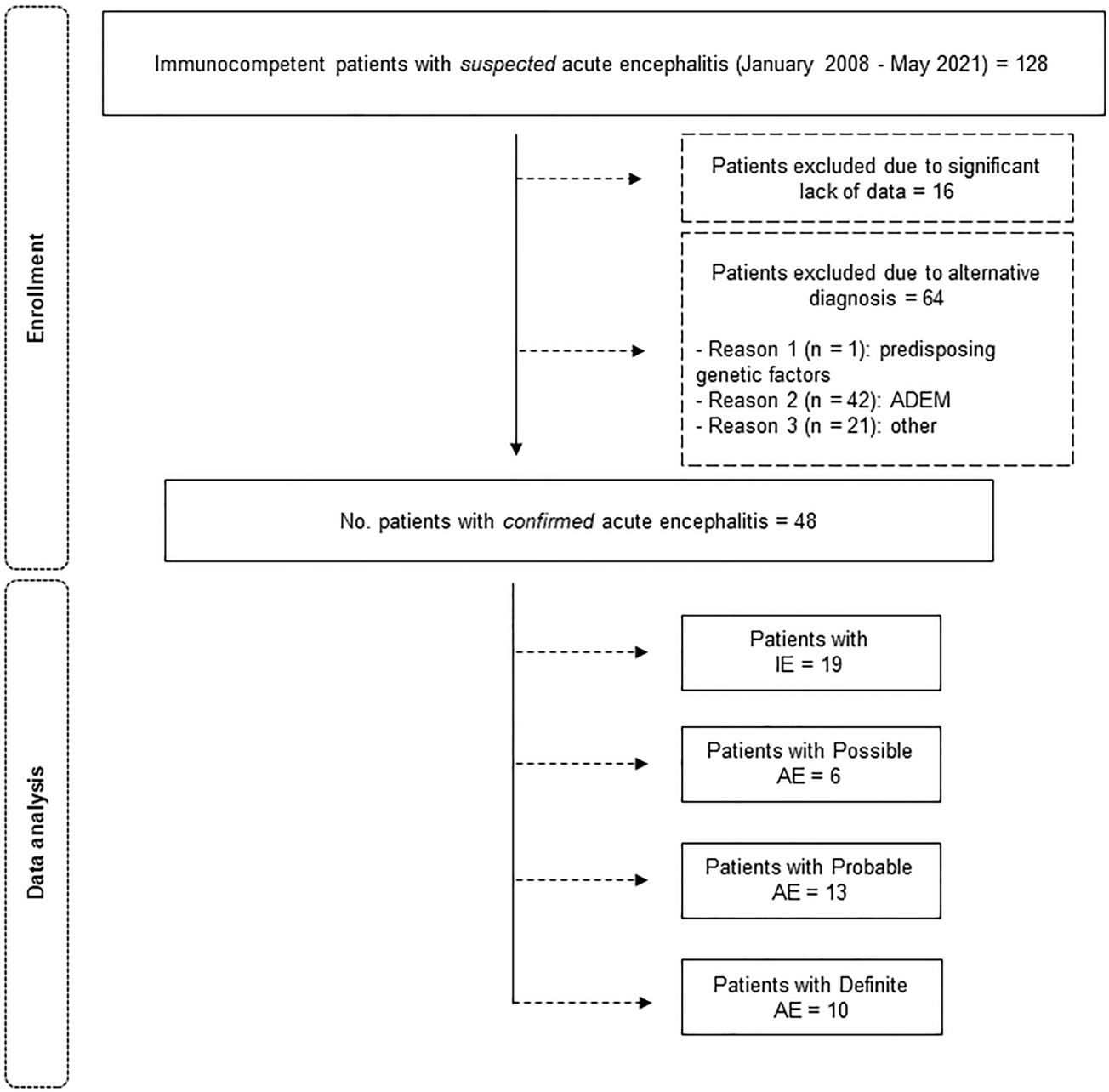



**Fig. 1** Flow-chart showing enrollment of immunocompetent patients with autoimmune encephalitis eligible for the study and exclusion of subjects due to alternative diagnosis or lack of required data (top on the right). Classification into infectious encephalitis (IE), possible, probable, and defined autoimmune encephalitis (AE) after clinical, laboratory, and instrumental data analysis

autoantibodies associated with AE. Patients with limbic encephalitis, with or without positive autoantibodies, were included in the Definite AE group, according to the adult criteria [4]. Children with possible or probable AE but preexisting developmental delay or chronic behavioral/psychiatric abnormalities were excluded from the study, since alternative diagnoses, such as genetic, metabolic, or neurodegenerative etiologies, should be considered for these patients.

Final diagnoses were determined by consensus.

## Standard of care

According to our Institutional protocol, children with suspected acute encephalitis underwent the following diagnostic tests:

– Blood tests: leukocytes, C reactive protein, blood cultures, serology, and PCR for Herpes simplex virus

(HSV) 1–2, CMV, Adenovirus, Epstein Barr Virus (EBV), CMV, VZV, Human Herpes virus (HHV) 6–7;
- CSF tests: cell count, proteins, glucose, gram stain, bacterial culture, PCR for HSV1-2, Adenovirus, EBV, CMV, VZV, HHV6, HHV 7, Enterovirus, Parechovirus, Borrelia burgdorferi, SARS-CoV-2 (since June 2020), and Mycoplasma pneumoniae, immunochemical test, and oligoclonal bands;
- Multiplex testing on throat swab with PCR for bacteria and viruses (Influenza A/B, Rhinovirus, RSV, Bocavirus, Metapneumovirus, SARS-CoV-2 since March 2020, Chlamydia pneumoniae, Mycoplasma pneumoniae, Streptococcus pneumoniae, Bordetella pertussis, Hemophilus influenza)
- Cell-based assays for neuronal autoantibodies on CSF and serum samples, included the following: NMDAR, LGI1, CASPR2, AMPAR1/2, and GABABR antibodies (Euroimmun, Germany), and tissue-based assay (indirect immunofluorescence on primate cerebellar tissue), and line-blot for GAD65 antibodies (both by Euroimmun, Germany).Brain MRI with structural sequences included diffusion-weighted (DWI), 3D T2-Fluid-Attenuated-Inversion-Recovery (FLAIR), T2-weighted turbo-spin-echo, 3D-T1-weighted, Susceptibility-weighted (SWI) images. The protocol included also post-contrast Fat-saturated 3D-T1-Turbo Spin Echo and Fat-saturated 3D-FLAIR images.

Given the retrospective nature of the study that covers several years in which new serological or CSF markers have been discovered and described, not all patients underwent all the diagnostic tests.

As for treatment, acyclovir at a dosage of 20 mg/kg q8h was administered empirically until definite exclusion of HSV encephalitis. First-line immunomodulatory treatment consisted of steroids, intravenous immunoglobulin (IVIG), or plasma exchange (PLEX) alone or in combination.

Early treatment was defined within 3rd day of admission for patients who received acyclovir and within 7th day of admission for patients who received first-line immunomodulatory treatment.

Due to the retrospective and not-homogeneous nature of the study, we decided to use the Expanded Disability Status Scale (EDSS) to quantify eventual residual disability. In fact, the EDSS is based on a neurological examination by a clinician, available for all subjects at the end of follow-up.

The post-acute rehabilitation period, the possible presence of cognitive impairment, motor disability, and/or residual epilepsy were also evaluated.

The outcome was evaluated globally and in relation to the timing of therapy starting.

## Statistical analysis

A descriptive analysis was performed, and the data were expressed as mean and standard deviation (SD) and median and range for continuous variables, and as absolute and relative frequencies for categorical variables. Given the limited size of the sample, it was considered appropriate to use a non-parametric statistical approach. Therefore, the comparisons were made using the Mann–Whitney test for the continuous variables.

The association between categorical variables was carried out using the $\chi 2$ test or Fisher's Exact Test when appropriate. For continuous variables, comparisons were made using analysis of variance (ANOVA).

A comparison was made between IE and AE globally. Because of the diagnostic uncertainty of possible AE, a comparison of differences between IE and definite plus probable AE was also performed excluding possible AE. For this second analysis, Bonferroni's rule was applied and the statistical significance was evaluated for a $p$-value $\leq 0.025$.

The statistical analysis was conducted using SPSS software version number 18 for Windows (SPSS Inc, Chicago, Illinois USA).

All tests were two-tailed and a $p$-value $\leq 0.05$ was considered statistically significant.

## Results

A total of 128 patients were evaluated at IGG between January 2008 and May 2021 for suspected acute encephalitis in immunocompetent hosts.

Sixteen patients were excluded from the study due to the significant lack of data that did not allow their analysis and other 64 due to an alternative final diagnosis (Fig. 1).

Forty-eight patients with a confirmed diagnosis of acute encephalitis (26 females, 22 males) were evaluated for the study. Among them, 19 were categorized as IE and 29 as AE. The median age at onset of symptoms was 4.4 (IQR 2.2–8) years.

In all patients with IE, a microbial pathogen strongly suspected as the cause of encephalitis was detected. Data are reported in Online Resource 1.

For Definite and Probable AE, the diagnosis was made by combining the clinical picture with CSF, neuroradiological or serological data, according to the newly proposed criteria (see: Methods, Online Resources 2 and 3).

More specifically, 41/48 (85%) patients underwent viral PCR testing on CSF while autoantibody assay on CSF was performed in 27/48 (56%) patients.

One patient had a previous diagnosis of medulloblastoma (39 months before encephalitis), and one other patient received a diagnosis of medulloblastoma 14 months after

encephalitis. Both are included in the group of probable encephalitis as paraneoplastic encephalitis (Online Resource 3).

Patients with AE were older than patients with IE ($5.5 \pm 4.3$ vs $3.2 \pm 2.9$ years, $p < 0.044$).

Fever was present in 89% and 62% of IE and AE, respectively ($p = 0.037$). The most reported neurologic symptoms were seizures (67%) and decreased consciousness (60%), similarly represented in both infectious and autoimmune forms. Notably, in the group of definite AE, seizures at onset were present in 80% of the patients. Status epilepticus and headache on admission were present in 21% and 31% of patients, respectively, and were equally represented among IE and AE.

Nineteen patients (40%) had a movement disorder with the majority belonging to the AE group (11% vs 63%, $p < 0.001$).

Further details on demographic characteristics and clinical symptoms are shown in Table 1.

## Neurologic imaging and EEG:

Overall, 35 brain CT scans were performed showing abnormalities (sub-cortical bilateral hypodense lesions) in two patients, one affected by IE (patient IE2 Online Resource 1) and one with probable AE (patient PrE7, Online Resource 3). Brain MRI was performed in 47 out of 48 (98%) patients with the detection of inflammatory abnormalities in 30 cases (63%). For details see Online Resources **1,2,3**; examples are shown in Fig. 2.

For patients with evidence of brain signal changes on MRI, further classification was made, dividing patients according to the location of the lesions (involvement of the cortex, basal nuclei, brainstem, and cerebellum), as shown in Fig. 3. Overall, no statistically significant correlation was found between the site of brain signal changes on MRI and the etiology.

However, the cortex was more frequently involved in patients with IE (57%) than in patients with AE (32%).

**Table 1** Demographic and clinical characteristics of patients with acute encephalitis

| | Acute encephalitis | IE | AE | p (IE vs AE) | Definite AE | Probable AE | Possible AE | p (Definite vs Probable) |
|---|---|---|---|---|---|---|---|---|
| Patients, *n* (%) | 48 (100) | 19 (40) | 29 (60) | | 10 (21) | 13 (27) | 6 (13) | |
| Age (median, y) | 4.4 | 3.2 | 5.5 | **0.044** | 4.3 | 4.3 | 8.8 | 0.100 |
| Sex | | | | | | | | 0.215 |
| Male | 26 (54) | 7 (37) | 19 (66) | 0.077 | 9 (90) | 5 (39) | 5 (83) | |
| Female | 22 (46) | 12 (63) | 10 (34) | | 1 (10) | 8 (61) | 1 (17) | |
| Family | | | | | | | | 0.146 |
| antecedents | 6 (13) | 1 (5) | 5 (17) | 0.184 | 4 (40) | 0 | 1 (17) | |
| Neurological | | | | | | | | |
| Immunological | 3 (6) | 0 | 3 (10) | | 2 (20) | 1 (8) | 0 | |
| Pre-existing neurological diseases | | | | 1.000 | | | | 1.000 |
| Seizure | 2 (4.2) | 0 | 1 (3) | | 0 | 1 (8) | 0 | |
| Psychomotor delay | 2 (4.2) | 0 | 1 (3) | | 0 | 0 | 1 (17) | |
| Recent vaccination (<1 month) | 7 (14.6) | 2 (10.5) | 5 (17) | 0.687 | 1 (10) | 4 (31) | 0 | 0.428 |
| Headache | 15 (31) | 6 (32) | 9 (31) | 1.000 | 4 (40) | 4 (31) | 1 (17) | 1.000 |
| Seizures | 32 (67) | 12 (64) | 20 (69) | 0.759 | 8 (80) | 9 (69) | 3 (50) | |
| Focal | 14 (29) | 8 (42) | 6 (21) | 0.075 | 5 (50) | - | 1 (17) | 0.516 |
| Generalized | 18 (37) | 4 (21) | | | | | | |
| | 14 (45) | | 3 (30) | 8 (62) | 2 (33) | 0.125 | | |
| Decreased | | | | | | | | 0.434 |
| Consciousness | 29 (60) | 11 (58) | 18 (62) | 0.225 | 5 (50) | 11 (85) | 2 (33) | |
| Coma | 6 (13) | 2 (11) | 4 (14) | 1.000 | 2 (20) | 2 (15) | - | 0.673 |
| Behavioral abnormalities | 9 (19) | 2 (10.5) | 7 (24) | 0.233 | 4 (40) | - | 3 (50) | 0.328 |
| Status epilepticus | 10 (21) | 4 (21) | 6 (21) | 1.000 | 2 (20) | 3 (23) | 1 (17) | 1.000 |
| Movement disorders | 19 (40) | 2 (11) | 17 (63) | **<0.001** | 5 (56) | 8 (67) | 4 (67) | **0.002** |
| Fever | 35 (73) | 17 (89) | 18 (62) | **0.049** | 3 (30) | 10 (77) | 5 (83) | **0.037** |
| Vomiting | 13 (27) | 4 (21) | 9 (31) | 0.762 | 2 (20) | 6 (46) | 1 (17) | 0.525 |

*IE* infectious encephalitis, *AE* autoimmune encephalitis, *y* years

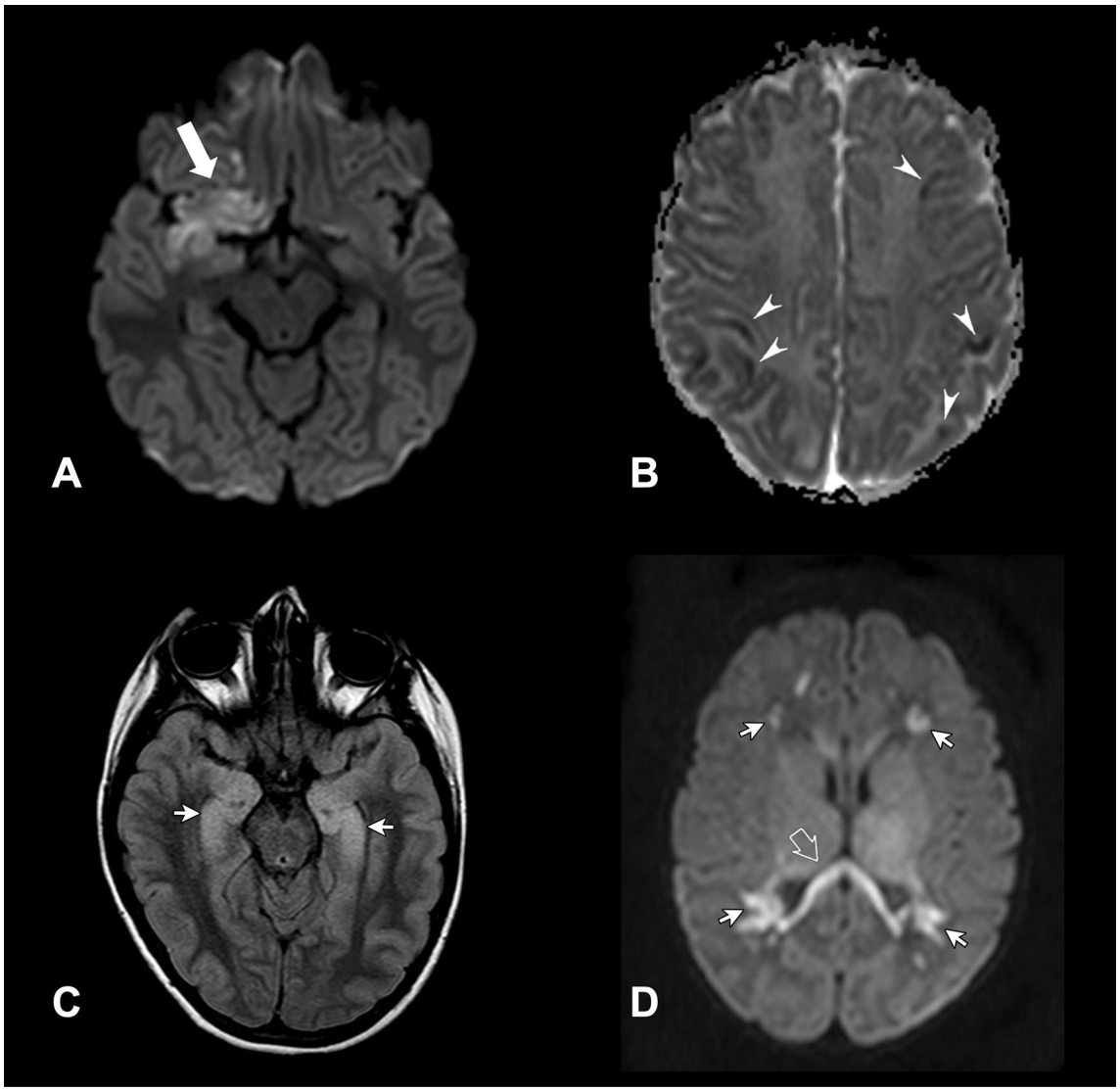



**Fig. 2** **A** A 8-year-old boy with herpes simplex virus encephalitis (HSV1) presented with fever and altered sensorium. Axial Diffusion-weighted imaging (DWI) shows restricted diffusion in the right frontotemporal region (White arrow). **B** A 15-day infant with herpes simplex virus encephalitis (HSV2) presented with fever and seizures. The axial Apparent Diffusion Coefficient map (ADC) shows a small area of restricted diffusion in multiple cortical regions in the frontal and parietal lobes (arrowheads). **C** A 9-years old girl with seronegative autoimmune limbic encephalitis presented with generalized seizures and decreased consciousness. Axial T2 FLAIR Image shows swelling and hyperintensities in both mesial temporal regions (white arrows). **D** A 21-day infant with parechovirus encephalitis presented with fever and focal seizures. Axial Diffusion-weighted imaging (DWI) shows multiple areas of restricted diffusion in the periventricular with matter (White arrows). Note the acute Wallerian degeneration of the splenium of the corpus callosum (empty arrow)

In particular, the temporal lobe and insula were more frequently involved in patients with IE (7/19; 32%) compared with AE (5/29; 17%). In the group of IE, 4 out of 7 subjects (57%) with temporal and insular lobes involvement presented HSV-1-related encephalitis. Frontal and parietal lobes were less frequently affected in both IE (2/19; 10%) and AE (3/29; 10%) groups. Moreover, the brainstem and cerebellum were rarely found to be the site of inflammatory changes on MRI. Contrast material was injected in 39 out of 48 subjects (15 IE and 24 AE). On MR images acquired at the acute stage of the disease, only 5 patients presented contrast enhancement within the affected region of the brain (3 IE and 2 AE). On SWI sequences, no patients showed signs of brain hemorrhages in both IE or AE groups.

An EEG during the acute phase was performed in 45/48 patients (94%) and abnormal findings were found in 87%. The most common pathological findings were slow abnormalities, present in 53% of IE and in 55% of AE (particularly in 70% of defined AE). Epileptiform abnormalities were

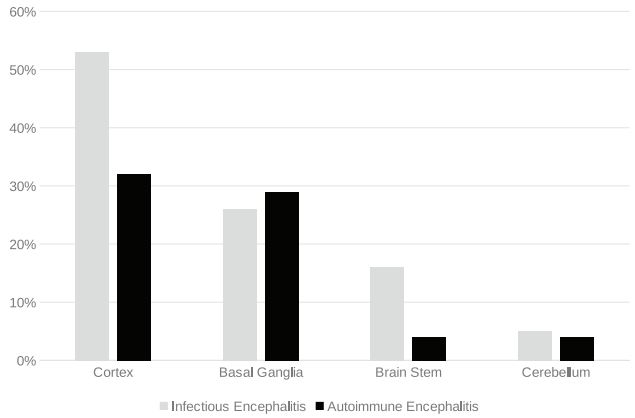



**Fig. 3** MRI lesion sites in patients with acute encephalitis

detected in 4/29 (14%) of patients with AE compared to 6/19 (32%) of those with IE. For details see Table 2.

## Etiology

Among IE, HSV 1–2 were the most frequently identified pathogen (8/19, 42%) followed by EBV in 3 cases (16%). Furthermore, 2 cases of Enterovirus (10%), 2 cases of Parechovirus (10%), and other 2 cases of HHV 7 (10%) encephalitis were identified. Influenza virus was detected in one patient (6%), and Mycoplasma pneumonia in the remaining one (6%).

Among the 29 AE, according to the above-mentioned criteria, 10 (34%) were classified as definite (including 6 patients positive for anti-NMDAR antibodies and 4 limbic encephalitis), 13 as probable (45%), and 6 as possible (21%).

## Laboratory

On admission blood tests showed a increased white blood cell count higher for IE than AE (IE mean value ± SD: 10,764 ± 4761, AE 8270 ± 4256, $p = 0.02$) as well as neutrophil count (IE mean value ± SD: 6307 ± 4315, AE 4779 ± 3277, $p = 0.05$). Median C reactive protein was only slightly increased in both categories.

A lumbar puncture was performed in 40 out of 48 (83%) patients. A pleocytosis was found more frequently in IE than AE (12/17, 63%, and 10/23, 34%, respectively), although not statistically significant.

Neuronal autoantibodies on paired CSF and serum samples were investigated in 27/48 (56%) patients with IE and in 29/48 (60%) with AE.

Significant positivity was found in 7 patients who received a diagnosis of definite AE. Six patients had anti-NMDAR and 1 anti-GAD65 autoantibodies in limbic encephalitis (Online Resource 2).

## Treatment

Table 3 shows details of the treatment data.

Empirical antiviral therapy with acyclovir was administered in 16 (84.2%) of 19 patients with IE and in 17 (58.6%) of 29 with AE. Among patients with IE, acyclovir was started before the 3rd day of admission in 15 cases (93.8%). Overall, 28 (58%) patients received empirical antibiotic treatment.

First-line immunomodulatory therapy (steroids, IVIG, or PLEX alone or in combination) was started in 13/19 patients (81%) with IE and 27/29 (93%) with AE.

First-line immunomodulatory therapy in patients with AE was started early (i.e. before the 7th day of admission) in 13/27 patients (48%).

Two patients (4%) affected by definite AE required a second- or third-line immunomodulatory therapy (rituximab, azathioprine, bortezomib), see Online Resource 2.

## Outcome and follow-up

As shown in Table 3, 16 patients (33%) required admission to the Pediatric Intensive Care Unit (PICU), with no difference between the two main groups.

In 6 (13%) patients (2 with IE, 2 with definite AE, and 2 with probable AE) the cause of PICU admission was status epilepticus.

The need for mechanical ventilation was higher for IE (37%) than for AE (17%), although not statistically significant ($p = 0.176$).

Notably, among the 5 patients with AE who needed mechanical ventilation, 4 were affected by definite AE.

One patient, affected by limbic encephalitis, died 3 months after onset.

The median length of stay was significantly longer for AE than IE patients (46 vs 13 days, respectively, $p = 0.001$).

The median follow-up was 34.5 months (IQR 5–52,5).

Functional recovery (assessed by an EDSS 0) was complete in 6 (32%) of IE and 12 (41%) of AE ($p = 0.554$).

Residual chronic epilepsy with seizures persisting after the acute phase was present in 17 patients overall (35%): 5 having had an IE (26%) and 12 (41%) with AE ($p = 0.363$).

At follow-up, 47% of patients with IE and 72% of patients with AE were taking antiepileptic therapy ($p = 0.177$).

Overall, a follow-up brain MRI was performed in 35 out of 45 (78%) patients resulting negative in 8 patients (23%), improved in 13 patients (37%), stable in 9 patients (26%), and worsened in 5 (14%).

Among IE, almost all patients who received acyclovir started the treatment before the 3$^{rd}$ day of admission (15/16, 94%).

Table 4 shows outcome data in relation to the timing of the immunomodulatory treatment for patients with AE.

**Table 2** Diagnostic tests performed in patients with acute encephalitis

| | Acute encephalitis | IE | AE | P (IE vs AE) | Definite AE | Probable AE | Possible AE | p (Definite vs Probable) |
|---|---|---|---|---|---|---|---|---|
| Patients, *n* (%) | 48 (100) | 19 | 29 | | 10 | 13 | 6 | |
| Blood PCR | 43 (89) | 15 (79) | 28 (96) | **0.007** | 9 (90) | 13 (100) | 6(13) | **0.023** |
| Negative | 27 (56) | 5 (26) | 22 (79) | | 6 (67) | 10 (77) | 6 (13) | |
| Positive | 16 (33) | 10 (53) | 6 (21) | | 3 (33) | 3 ( 23) | - | |
| CSF PCR | 41 (85) | 18 (95) | 23 (79) | **<0.001** | 8 (80) | 10 (77) | 5 (83) | **<0.001** |
| negative | 31 (64) | 8 (42)) | 23 (100) | | 8 (80) | 10 (77) | 5 (83) | |
| Positive | 10 (21) | 10 (53 | - | | - | - | - | |
| Serology | 45 (94) | 18 (95) | 27 (93) | **<0.001** | 9 (90) | 12 (92) | 6 (100) | **<0.001** |
| Negative | 31 (65) | 4 (21) | 27 (100) | | 9 (100) | 12 (100) | 6 (100) | |
| Positive | 14 (29) | 14 (74) | - | | - | - | - | |
| Multiplex | | | | **0.032** | | | | **0.033** |
| respiratory | 34 (71) | 13 (68) | 21 (72) | | 8 (80) | 9 (69) | 4 ( 67) | |
| negative | 17 (35) | 3 (16) | 14 (67) | | 6 (75) | 5 (56) | 3 (75) | |
| positive | 17 (35) | 10 (53) | 7 (33) | | 2 (25) | 4 (44) | 1 (25) | |
| Lumbar | | | | | | | | |
| Puncture | 40 (83) | 17 (89) | 23 (88) | 0.116 | 8 (80) | 10 (77) | 5 (10) | 0.489 |
| Pleocytosis | 22 (46) | 12 (63) | 10 (34) | 0.425 | 4 (50) | 6 (60) | - | 0.723 |
| High protein level | 12 (25) | 6 (32) | 6 (21) | | 1 (13) | 5 (50) | - | |
| CSF | | | | | | | | 0.521 |
| autoantibodies | 27 (56) | 3 (16) | 24 (83) | 0.545 | 10 (100) | 8 (62) | 6 (100) | |
| Negative | 20 (42) | 3 (16) | 17 (71) | | 3 (30) | 8 (100) | 6 (100) | |
| Positive | 7 (15) | - | 7 (29) | | 7 (70) | - | - | |
| Serum autoantibodies | 29 (60) | 5 (26) | 24 (83) | 0.296 | 10 (100) | 8 (62) | 6 (100) | 0.272 |
| Negative | 22 (76) | 5 (26) | 17 (59) | | 3 (30) | 8 (100) | 6 (100) | |
| Positive | 7 (24) | - | 7 (24) | | 7 (70) | - | - | |
| Brain CT | 35 (73) | 12 (63) | 23 (79) | 0.670 | 8 (80) | 10 (77) | 5 (83) | 0.660 |
| Normal | 28 (58) | 11(58) | 22(76) | | 8(80) | 8(61) | 5 (83) | |
| Pathological | 2 (41) | 1 (5) | 1 (3) | | - | 1 (8) | - | |
| Brain MRI | 47 (98) | 19(100) | 28 (97) | | 9 (90) | 13 (100) | 6 (100) | 0.325 |
| Normal | 17 (36) | 4(21) | 13 (46) | 0.122 | 6 (67) | 2 (15) | 5 (83) | |
| inflammatory changes | 30 (64) | 15(79) | 15 (53) | | 3 (33) | 11 (85) | 1 (17) | |
| Lesions site | | | | 0.396 | | | - | 0.396 |
| cortical | 11 (23) | 7(47) | 4(31) | | 1(50) | 3(27) | - | |
| subcortical | 10 (21) | 6 (40) | 4 (31) | | - | 4 (36) | - | |
| Both | 7 (15) | 2 (13) | 5 (39) | | 1 (50) | 4 (36) | | |
| Cortex involvement | | | | | | | | 0.538 |
| no | 28 (60 | 9(47.4) | 19(68) | 0.228 | 7(78) | 6(46) | 6(100) | |
| Yes | 19 (40) | 10 (53) | 9 (32) | | 2 (22) | 7 (54) | - | |
| Basal ganglia | | | | | | | | 0.524 |
| No | 34 (72) | 14(74) | 20(71) | 1.000 | 8(89) | 6(46) | 6(100) | |
| Yes | 13 (28) | 5 (26) | 8 (29) | | 1 (11) | 7 (54) | - | |
| Brain Stem | | | | | | | | 0.321 |
| No | 43 (91) | 16(84.2) | 27(95) | 0.289 | 9(100) | 12(92) | 6(100) | |
| Yes | 4 (9) | 3 (16) | 1 (4) | | - | 1 (8) | - | |
| Cerebellum | | | | | | | 6(100) | 1.000 |
| No | 45 (96) | 18 (95) | 27 (96) | 1.000 | 9(100) | 12 (92) | - | |
| Yes | 2 (4) | 1 (5) | 1 (4) | | - | 1 (8) | | |
| DWI restrictions | | | | | | | | 0.355 |

**Table 2** (continued)

| | Acute encephalitis | IE | AE | P (IE vs AE) | Definite AE | Probable AE | Possible AE | p (Definite vs Probable) |
|---|---|---|---|---|---|---|---|---|
| No | 29 (62) | 9(47) | 20(71) | 0.130 | 9(100) | 5(39) | 6(100) | |
| Yes | 18 (38) | 10 (53) | 8(28.6) | | - | 8 (62) | - | |
| Arterial spin labeling | 27 (56) | 5 (26) | 22 (75) | 0.437 | 1 (11) | 4 (31) | 1 (17) | 0.580 |
| normal- | 7 (15) | 2 (11) | 5 (18) | | 1 (11) | 3 (23) | 1 (17) | |
| Hypoperfusion | 3 (6) | 2 (11) | - | | - | 1 (8) | - | |
| Hyperperfusion | 1 (2) | 1 (5) | 1 (4) | | - | - | - | |
| EEG | 45 (94) | 19 (100) | 26 (90) | 0.249 | 10 (100) | 10 (77) | 6 (100) | 0.432 |
| Normal | 6 (13) | 1 (5) | 5 (17) | 0.465 | 1 (10) | 2 (15) | 2 (33) | |
| Slow abnormalities | 26 (58) | 10 (53) | 16 (55) | | 7 (70) | 5 (39) | 4 (68) | |
| Epileptic abnormalities | 10 (22) | 6 (32) | 4 (14) | | 2 (20) | 2 (15) | - | 0.711 |
| Delta activity | 3 (7) | 2 (11) | 1 (3) | | - | 1 (8) | - | |

*IE* infectious encephalitis, *AE* autoimmune encephalitis, *CSF* cerebrospinal fluid, *PCR* polymerase chain reaction, *CT* computed tomography, *MRI* magnetic resonance imaging, *DWI* diffusion-weighted imaging

**Table 3** Treatment and outcome of patients with acute encephalitis

| | Acute encephalitis | IE | AE | p (AE vs IE) | Definite AE | Probable AE | Possible AE | p (Definite vs probable AE) |
|---|---|---|---|---|---|---|---|---|
| Patients, n (%) | 48 (100) | 19 (40) | 29 (60) | | 10 (21) | 13 (27) | 6 (13) | |
| Antibiotic | 18 (58) | 13 (68) | 15 (54) | 0.374 | 4 (40) | 8 (66.7) | 3 (50) | 0.522 |
| Acyclovir | 33 (69) | 16 (84) | 17 (59) | 0.213 | 6 (60) | 9 (69) | 2 (33) | 0.495 |
| Acyclovir < 3 days | 28 (58) | 15 (94) | 13 (65) | **0.053** | 5 (62) | 6 (60) | 2 (100) | **0.043** |
| Immunomodulant treatment | 41 (95) | 13 (81) | 28 (97) | 0.512 | 10 (100) | 12 (92) | 6 (100) | 1.000 |
| Immunomodulant treatment ≤ 7 days | 23 (56) | 11 (85) | 12 (41) | **0.005** | 4 (43) | 6 (50) | 2 (33) | **0.011** |
| Second/third line immunomodulant treatment | 2 (4) | - | 2 (7) | 1.000 | 1 (10) | – | 1 (17) | 1.000 |
| ICU admission | 16 (33) | 7 (37) | 9 (31) | 0.759 | 5 (39) | – | 9 (31) | 1.000 |
| Length of stay, mean (days) | 34 | 14 | 46 | **< 0.001** | 37 | 48 | 50 | **< 0.001** |
| Mechanical ventilation | 12 | 7 (37) | 5 (17) | 0.176 | 4 (40) | 1 (8) | – | 0.323 |
| Death | 1 (2) | – | 1 (3) | 1.000 | 1 (10) | – | – | 1.000 |
| Complete functional recovery | 18 (38) | 6 (32) | 12 (41) | 0.554 | 3 (30) | 5 (38) | 4 (66) | 1.000 |
| Residual cognitive impairment | 19 (40) | 5 (26) | 14 (48) | 0.146 | 7 (70) | 4 (31) | 3 (50) | 0.337 |
| Residual motor deficit | 22 (46) | 8 (42) | 14 (48) | 0.555 | 5 (50) | 6 (46) | 3 (50) | 0.542 |
| Residual epilepsy | 17 (35) | 5 (26) | 12 (41) | 0.363 | 5 (50) | 5 (38) | 2 (33) | 0.337 |
| Anti-seizure medications | 30 (62) | 9 (47) | 21 (72) | 0.127 | 8 (80) | 8 (61) | 5 (83) | 0.209 |
| Post-acute rehabilitation | 26 (54) | 8 (42) | 18 (62) | 0.239 | 8 (80) | 7 (54) | 3 (50) | 0.213 |

*IE* infectious encephalitis, *AE* autoimmune encephalitis, *ICU* Intensive Care Unit

Among patients with AE who received first-line immunomodulatory therapy, patients who started therapy after 7 days had a statistically significant lower rate of complete functional recovery ($p=0.002$). This was also true considering only the group of definite and probable patients. ($p=0.031$). Furthermore, they showed more frequent residual motor and cognitive deficit (64% vs 36% in both cases), required post-acute rehabilitation more often (61% vs 39%), and had a higher incidence of residual epilepsy (58% vs 42%) at follow-up, although these findings did not reach a statistical significance.

**Table 4** Outcome data in relation to the timing of the immunomodulatory treatment for patients with autoimmune encephalitis

| | Immunomodulatory < 7 days | Immunomodulatory > 7 days | p |
|---|---|---|---|
| Complete functional recovery | | | |
| Autoimmune | 11 (92) | 1 (8) | **0.002** |
| D + P | 7 (88) | 1 (12) | **0.031** |
| Residual cognitive impairment | | | |
| Autoimmune | 9 (64) | 5 (36) | 0.704 |
| D + P | 5 (50) | 5 (50) | 1.000 |
| Residual motor deficit | | | |
| Autoimmune | 9 (64) | 5 (36) | 0.704 |
| D + P | 6 (55) | 5 (45) | 1.000 |
| Residual epilepsy | | | |
| Autoimmune | 7 (58) | 5 (42) | 1.000 |
| D + P | 5 (50) | 5 (50) | 1.000 |
| Anti-seizure medications | | | |
| Autoimmune | 11 (55) | 9 (45) | 1.000 |
| D + P | 8 (53) | 7 (47) | 1.000 |
| Post-acute rehabilitation | | | |
| Autoimmune | 11 (61) | 7 (39) | 0.698 |
| D + P | 8 (53) | 7 (47) | 1.000 |

*D* definite, *P* probable

## Discussion

Encephalitis is a rare but severe condition due to inflammation of the brain parenchyma which also in the pediatric population may result in substantial morbidity, disability, and even mortality. A wide variety of infectious and noninfectious etiologies are associated with encephalitis, though the cause in more than half of cases remains unexplained despite extensive testing [2, 6].

Autoimmune encephalitis is a potentially curable illness whose awareness has significantly grown in recent years. Due to its potentially treatable etiology, early recognition of AE is crucial in real-world clinical practice [10]. Since the clinical picture in AE is heterogeneous, autoantibodies can be negative and immunomodulatory treatments can be sometimes ineffective, new diagnostic criteria were defined by consensus in 2016 [4] and later proposed for pediatric ages in 2020 [5]. We decided to review all the patients evaluated for acute encephalitis in our Hospital, classifying them as infectious or autoimmune and subsequently applying to a real-world setting the new proposed pediatric criteria thus subdividing AE into definite, probable, and possible. According to the adult diagnostic criteria [4] we considered limbic encephalitis as belonging to the group of definite AE.

Overall, we had 19 (40%) patients with IE and 29 (60%) with AE. Putting aside patients with probable ($N = 13$) and possible ($N = 6$) autoimmune etiologies (groups in which other diagnoses can be hidden) we observed that infections were the most frequently diagnosed causes of encephalitis compared to definite autoimmune (19 versus 10).

IE was specifically frequent in children < 5 years, while autoimmune encephalitis was more often seen in older children.

Among IE, HSV (8/19, 42%), EBV (3/19, 16%), enterovirus (2/19, 10%), and parechovirus (2/19, 10%) were the most frequent pathogens detected. In spite of the controversy regarding the role of EBV in immunocompetent individuals, we decided to classify the 3 patients with EBV encephalitis in relation to IgM concomitant positivity and/or a clinical picture of acute primary EBV infection with the exclusion of other known causes.

Although enterovirus is currently reported as the second most common cause of encephalitis in children [2], it seems to be underreported in our cohort. Enteroviruses are RNA viruses, meaning that the genetic stability of the viral RNA is reduced over time and the sensitivity of the enterovirus CSF PCR may be low when CSF samples are not collected early, i.e., in the first 3 days of the disease [11]. According to this, in cases with a late presentation, the presence of enteroviruses should be investigated in different body sites, such as rectal/stool swabs, nasopharyngeal specimens, and/or blood [11]. Likely this practice was not performed routinely in all patients in our cohort, thus accounting for an inappropriately lower rate of enterovirus infection.

With the limits of our classification, we have considered two cases with positivity to HHV7, presenting with fever and no other clear cause of infection/inflammation, as HHV7 encephalitis, thus belonging to the group of IE. HHV-7 has been associated with encephalitis in immunocompromised patients or in coinfections with other viruses, while encephalitis due to HHV7 is considered rare in immunocompetent children [12, 13]. As HHV-7 is ubiquitous and establishes lifelong latency, it is often difficult to interpret the clinical relevance of HHV-7 detection in the CNS. HHV-7 DNA in the CSF alone is considered insufficient to prove an etiologic association of encephalitis in immunocompetent children [14]. It may be considered in cases when no alternative cause is identified and, ideally, it should be combined with a serological response to HHV-7 [14]. Among the 10 patients with definite AE, 6 had anti-NMDAR antibodies and 4 had limbic encephalitis, one of them with anti-GAD65 antibodies.

Among patients with probable AE, we had two patients with paraneoplastic encephalitis, both associated with a diagnosis of medulloblastoma before or after (within 4 years) encephalitis.

Immunomodulatory treatment was started 7 days from the onset of symptoms and both patients required ICU

admission and had residual epilepsy and significant functional sequelae.

As for clinical features, our data confirm that fever is a common symptom in patients with acute encephalitis (73% in our cohort), and more often accompanies the onset of symptoms in infectious forms ($p = 0.049$). In our cohort, the occurrence of movement disorders was highly suggestive of an autoimmune etiology and, in particular of anti-NMDA receptor encephalitis ($p < 0.001$). Although movement disorders are also reported in association with viruses with tropism for basal ganglia (i.e. west nile virus, japanese encephalitis virus) [15, 16], our findings are in line with the literature [17, 18]. A previous study found that the antibody-mediated NMDAR decreases inactivated GABA-ergic neurons, therefore, leading to movement disorders [19].

Conversely, the presence of seizures, status epilepticus, or headache, was not different between the two main groups. Interestingly, even if not statistically significant, seizures were a frequent sign of onset (80%) in definite AE, as very commonly observed in anti-NMDAR and limbic encephalitis.

Considering neuroimaging findings, brain MRI identified brain abnormalities more frequently compared to CT scans, with 63% of MRI imaging showing brain signal changes, mostly involving the cerebral cortex. No statistically significant differences in MRI findings were found between patients with IE and AE, nor comparing IE versus definite/probable AE. In our cohort of patients, brain MRI played a crucial role in diagnosing acute encephalitis, as it demonstrated brain signal abnormalities and contrast enhancement that reinforced the clinical suspicion of encephalitis, especially when the identification of the causal agent by lumbar puncture was pending. Moreover, the most severe neuroimaging findings were closely related to the severity of acute illness and the time of recovery. However, MRI patterns frequently required further revisions in light of the clinical context (immunosuppression, recent travel) and according to the course of the disease, contemplating additional differential diagnoses, mainly related to inflammatory, neoplastic, and ischemic causes. The use of advanced MRI sequences (i.e. advanced diffusion techniques and brain perfusion study) in the diagnostic workup of patients with encephalitis could improve the diagnostic accuracy of neuroimaging, especially in the early stages of the disease, since they can provide additional information which can help to elucidate the pathophysiological mechanisms of brain damage [20, 21].

In particular, perfusion-weighted images may have a promising role in identifying the regions of the brain affected by inflammation, especially in cases with normal findings at structural brain MRI. Indeed, in some patients with acute encephalitis, perfusion changes have been demonstrated even before the lesions were identifiable on both conventional structural and diffusion MRI sequences [22]. The exact mechanism of focal changes of perfusion in acute encephalitis is not well understood but the possibility of loss of cerebral vascular autoregulation has been suggested during the acute stage of encephalitis [23].

Similarly, EEG abnormalities, more often slow wave activity, were not different among the groups. Considering initial diagnostic tests, only the blood PCR and serology positivity, as well as for CSF PCR positivity, as expected, were statistically more frequent in patients with IE, while on blood tests patients with IE showed a higher white cell count compared to AE.

Autoantibodies were searched for on CSF and serum in more than half of patients (27/48, 56% and 29/48, 60% respectively); their positivity allowed, in association with the clinic-neuroradiological picture, diagnosis of definite AE in 7 cases (6 anti-NMDAR encephalitis and 1 anti-GAD65 limbic encephalitis).

Sixteen (33%) patients with acute encephalitis required PICU admission, and in 37% of cases, the cause was status epilepticus.

There were no differences between the two main groups in terms of severity, except for the significantly longer mean length of stay for AE than for IE ($p < 0.001$).

As known, the delay in starting treatment in the case of herpetic encephalitis is one of the main negative prognostic factors. In our patients, treatment with acyclovir was given to 84% (16/19) of children with IE, in almost all cases (15/16) before the 3$^{rd}$ day from admission.

Similarly, results from large studies have shown a statistically significant association between a good outcome and a lower relapse rate with the use of immune therapy in patients with autoimmune encephalitis, especially when started early in the course of the disease [24–26].

First-line immunotherapy was started in 97% of children with AE in our cohort, although only 45% had an early commencement (< 7th day from admission). Patients with AE (definite, probable, and possible) who started therapy after 7 days from the onset had a statistically significant lower rate of complete functional recovery ($p = 0.002$), while we observed no differences for residual epilepsy, need for anti-seizure medication, or post-acute rehabilitation. Only two patients with AE required a second- or third-line immunomodulatory treatment, one with anti-NMDAR encephalitis and one with anti-GAD65 limbic encephalitis. Among anti-NMDAR encephalitis, we had three patients (50%) who achieved complete functional recovery, while all patients with limbic encephalitis had neurological sequelae, in 75% of cases associated with residual epilepsy, one deceased.

Motor or cognitive deficits were quite common (between 26 and 48%) in all groups, without differences among groups, such as for the risk for residual epilepsy.

Notably, a substantial proportion (81%) of patients with an ultimate diagnosis of IE received immunomodulatory therapies.

The use of corticosteroids, IVIG, and PLEX has not been systematically studied with controlled trials in IE and the level of evidence is not strong enough to draw definite conclusions.

In particular, the role of corticosteroids remains debated and still undefined and is often related to local protocol and experiences. They can be given for cerebral edema and to reduce inflammation although if used in the early phase of viral replication, they may potentially weaken the immune system and lead to uncontrolled viral replication and permanent damage.

Currently, the use of corticosteroids is suggested, following a careful assessment of the risks and benefits, in some conditions such as EBV and VZV encephalitis [27], while some evidence is emerging about the potential efficacy of an additional course of corticosteroids started after the antiviral treatment also in HSV encephalitis [28]. A multinational, randomized, controlled trial (DexEnceph; ClinicalTrials.gov identifier NCT03084783) evaluating the benefit of administering intravenous dexamethasone in adults suffering from HSV encephalitis is ongoing.

In our experience, we treated 13 patients with IE (68%) with corticosteroids, and the decision to start steroid treatment was mainly driven by the neuroradiologic picture (edema, inflammation) or as an empirical approach following the concern for worsening conditions.

However, we suggest being cautious when considering immunomodulatory treatment until the potential for active infections has been adequately assessed or is being treated with effective antimicrobial therapy.

Mortality in this study (2%) was similar to or slightly lower than those reported in previous studies [3, 29, 30]. Over a mean period of 34.5 months after the hospital discharge, more than half of patients (62%) had an incomplete functional recovery (assessed using the EDSS), and this rate was similarly noted across the different groups. This is in line with the findings of large recent studies evaluating the long-term outcome of childhood encephalitis which reported incomplete recovery in 55–60% of cases [31, 32].

Our study has some limits: given the retrospective nature of the study which covers several years in which new serological or CSF markers and new criteria for diagnosis and classification have been proposed, not all patients underwent the same serological, CSF, and instrumental investigations, as reported in the "Methods". For some patients, some data are lacking from the retrospective evaluation of the available clinical records, as reported in the supplementary materials.

For all these reasons, it is possible that encephalitis of unknown cause, or "possible autoimmune encephalitis" is viral in origin (with specific viral pathogen not tested) or probable or definite autoimmune but with no sufficient neuroradiological or serological data to support a more precise diagnosis. Moreover, it is possible that in the group of possible AE, some patients have different etiologies. For this reason, we performed a statistical analysis comparing both IE and AE and then IE versus definite and probable AE, thus excluding the most confounding group.

Finally, the small size of our cohort and the single-center nature of the study limits the generalizability of our findings.

In conclusion, we have shown a detailed analysis of the acute phase and follow-up of a cohort of immunocompetent children managed for acute encephalitis at a tertiary referral children's hospital.

IE and AE are symptomatically similar and there are no clear clinical, or neuroradiological features to differentiate the different groups in the early stage of the disease.

Children with IE had more frequent fever and higher white blood cell count at onset, while AE were associated with older age, the occurrence of movement disorders, and a longer hospital stay.

Early initiation of immunomodulatory therapy in patients with autoimmune encephalitis improves clinical outcomes.

New approaches to early distinguishing among the different etiology of childhood encephalitis are required, including the use of next-generation sequencing that appears promising [33].

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**Data availability** Data of this study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The study was approved by the Regione Liguria Ethical Board and was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments.

**Informed consent** Informed consent was obtained from all partecipants or their parents.

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