Autoimmune encephalopathies in children: diagnostic clues and therapeutic challenges

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ABSTRACT

Neuronal surface antibody syndromes (NSAS) encompass a variety of disorders associated with “neuronal surface antibodies”. These share clinical and neuroradiological features that pose challenges related to their recognition and treatment. Recent epidemiological studies show a clear predominance for the glutamate-N-methyl-D-aspartate receptor encephalitis in both adults and pediatric population. Despite this, the overall NSAS’s incidence remains underestimated, and diagnosis persists to be not always easy to achieve. Based on current literature data, in this paper the authors propose a diagnostic pathway to approach and treat pediatric NSAS. An autoimmune etiology can be suggested through the integration of clinical, immunological, electrophysiological and neuroradiological data. On that basis, a target treatment can be started, consisting of corticosteroids and intravenous immunoglobulin or plasma exchange as a first-line immunotherapy, followed by second-line drugs including rituximab, cyclophosphamide or mycophenolate mophetil, if the case. In children a prompt diagnosis and a targeted treatment may lead to a better clinical outcome. Nevertheless further studies are required to assess the need of more tailored treatments according to long-term outcome findings and prognostic factors in different NSAS.

Key words: Autoimmune encephalitis; children; diagnosis

INTRODUCTION

Over the last decade there has been an increase in the identification of forms of encephalitis associated with “neuronal surface antibodies” (NSAbs). These have been labelled as “neuronal surface antibody syndromes” (NSAS).[1]

NSAS differ from encephalitis due to antibodies directed against intracellular neuronal antigens for a different etiopathogenetic mechanism, a weaker association with paraneoplastic syndromes, a better response to immunotherapy and a higher incidence in the pediatric population.[2-6] Pathogenesis predominantly involves humoral immune response, while cellular immune response activation may coexist in a variable proportion, according to the different forms. Target antigens include proteins with various roles in neuronal function, ranging from synaptic transmission and plasticity to ions channels’ clustering and modulation, and including also glutamic acid decarboxylase (GAD) enzyme when exposed on cellular surface during exocitosis.[1,7]

A number of studies reporting NSAS in infancy suggest that, so far, their incidence has been probably underestimated, due to the fact that they are still often unrecognized or identified at a later stage.[8,9]

In pediatric forms, as in adults one, females can be over-represented, and a history of other antibody-mediated condition is easily detectable.[10] Conversely, in children rather than adults, a paraneoplastic cause is less probable and the role of fever or intercurrent infections in supporting the autoimmune process is less clear.[1,10]
### Table 1: Details of NSAS encephalitis in adults and children

<table>
<thead>
<tr>
<th>Target</th>
<th>Patients</th>
<th>Clinical signs</th>
<th>Adult forms</th>
<th>Neuroimaging</th>
<th>Adult EEG pattern</th>
<th>Tumor association (%)</th>
<th>Outcome</th>
<th>Pediatric forms</th>
<th>Peculiar symptoms</th>
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<tbody>
<tr>
<td>Glutamate-N-methyl-D-aspartate receptor (NMDA-R)</td>
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<tr>
<td>Glutamate-N-methyl-D-aspartate receptor subunit 1</td>
<td>GluR1</td>
<td>F 80%</td>
<td>Age 1-80 (median 20 y)</td>
<td>Psychosis or epileptic seizures followed by insomnia, amnesia, MD, catatonia, AI, coma</td>
<td>Normal (50%)</td>
<td>-GS/FS (temporal) and bilateral (temporal)</td>
<td>Age dependent</td>
<td>Variable response to IM</td>
<td>Seizures &gt; psychosis occurrence (age dependent) EEG: Rarely extreme delta train</td>
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<tr>
<td>Glutamate-N-methyl-D-aspartate receptor subunit 2</td>
<td>GluR2</td>
<td>F 80%</td>
<td>Age 1-80 (median 20 y)</td>
<td>Psychosis or epileptic seizures followed by insomnia, amnesia, MD, catatonia, AI, coma</td>
<td>Normal (50%)</td>
<td>-GS/FS (temporal) and bilateral (temporal)</td>
<td>Age dependent</td>
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| Voltage-gated potassium channel (VGK) complex | | | | | | | | | |
| Voltage-gated potassium channel | VGKC | | | | | | | | |
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| Voltage-gated potassium channel | VGKC | | | | | | | | |

| Leucine-rich, glioma inactivated-1 component | LG1 | M 65% | Age 1-30 (median 60 y) | Prodromal hallucobrachial seizures, short-term memory loss, myoclonus, toxicosis, myoclonus | Normal (especially in Morvan's syndrome) | -GS/FS (temporal) and bilateral (temporal) | Age dependent | Monophasic with complete remission | Monophasic with complete remission |

| Contactin-associated protein-like 2 | CASPR2 | M 85% | Age 1-80 (median 60 y) | Morvan's syndrome; seizures, seizures, memory impairment hyponatremia (60%) | Normal | -GS/FS (temporal) and bilateral (temporal) | Age dependent | Monophasic with complete remission | Monophasic with complete remission |

| Alpha-alpha-3-hydroxy-5-methyl-4-isoxazolepropionic acid-glutamate receptor (AMPA-R) | GluA1/2 | F 40% | Age 1-80 (median 50 y) | Seizures, short term memory loss, disorientation, psychiatric, Frequent coexisting autonomic disturbances. | LE | -GS/FS (temporal) and bilateral (temporal) | Age dependent | Monophasic with complete remission | Monophasic with complete remission |

| Metabotropic glutamate receptors | Type 1 | mGluR1 | F 100% | Age 20-50 y | Cerebellitis | Normal (generically) | Normal | Monophasic or chronic disease course (correlation with titer). | Monophasic or chronic disease course (correlation with titer). |
| Type 2 | mGluR5 | F 50% | Age 15-45 y | Ophelia syndrome | FS (bi-temporal) or FIED, FIED or GED | HL (70%) | None | Monophasic or chronic disease course (correlation with titer). | Monophasic or chronic disease course (correlation with titer). |

| Gamma-amino-butyric acid receptors (GABA-B-R and GABA-A-R) | GABA_B-R associated protein (clustering and anchoring receptors) | GABARAP | M 60% | Age 2-74 y (median 22 y) | SEERS, encephalopathy, RS, SPS, CA | LE | -GS or GED/PSD and FIED | None | Variable | Seizures > psychosis occurrence (age dependent) EEG: Rarely extreme delta train |

| GABA_B-R subunit 1/2 | GABAB1 | F 50% | Age 25-75 y (median 60 y) | Prominent seizures (FS, GS), short term memory loss, psychosis | LE (often asymmetric) | -GS or GED/PSD and FIED | None | Variable | Seizures > psychosis occurrence (age dependent) EEG: Rarely extreme delta train |

| Dopamine receptor | Type 2 | D2-R | M 50% | Age 6-31 y (median 20 y) | SydCh, PANDAS, TS, encephalopathy, psychoses, psychiatric abnormalities | T2/FLAIR Basal ganglia abnormalities | Generally GS | Exceptional | Monophasic or relapsing remitting; good response to IM | Monophasic or relapsing remitting; good response to IM |

| Glycine receptor (Gly-R) | Gly-R subunit 1 | Gly1 | M 80% | Age 30-40 y (median 50 y) | Prominent PERM and SPS; hyperekplexia, tremor, My, caudoputamen, FS, brainstem dysfunction (rare) | Normal (ca. 70%) | LE or unspecified (rare) | Normal | Monophasic or chronic disease course (correlation with titer). | Monophasic or chronic disease course (correlation with titer). |

| Glycine receptor (Gly-R) | Gly-R subunit 3 | Gly3 | M 80% | Age 10-20 y (median 14 y) | Prominent PERM and SPS; hyperekplexia, tremor, My, caudoputamen, FS, brainstem dysfunction (rare) | Normal (ca. 70%) | LE or unspecified (rare) | Normal | Monophasic or chronic disease course (correlation with titer). | Monophasic or chronic disease course (correlation with titer). |

| Dipeptidyl-peptidase-like protein 6 | Cell surface auxiliary subunit of the K2P-2 potassium channel | DPPX | M 80% | Age 13-75 y (median 53 y) | Prodromal severe gastrointestinal dysfunction, encephalopathy, agitation, hallucinations, tremor, PERM, My, startle with muscle rigidity, sleep disturbances, Al seizures (rare) | Normal | -Asymmetrical T2/FLAIR abnormalities (rare) | Normal | None | Seizures > psychosis occurrence (age dependent) EEE: Rarely extreme delta train |

| Neuronal cell adhesion molecule (LgLNS) | Neuronal cell adhesion molecule protein (LGNC) | LGNC | M 50% | Age 52-78 y (median 59 y) | Prominent sleep dysfunction, abnormal sleep movements, OSAS, Cho, CA, progressive memory loss, mild gast oesophageal reflux | Normal | Normal | None | Chronic disease course refractory to IM | Seizures > psychosis occurrence (age dependent) EEE: Rarely extreme delta train |

| Glutamic acid-decarboxylase | Glutamic acid-decarboxylase 6 (GAD6) | GAD6 | F 80% | Age 15-80 y (median 70 y) | Neurocognitive disorders, LE, TLE with RRE, SPS, PERM, CA, DM1 (if titer < 20 U/ml) Frequent coexisting autonomic disturbances | Normal | -LE or mesiotemporal brain stem, cerebellum and spiral cord T2/FLAIR abnormalities evolving into atrophy. | Normal | None | Seizures > psychosis occurrence (age dependent) EEE: Rarely extreme delta train |

| Glutamic acid-decarboxylase | Glutamic acid-decarboxylase 5 (GAD5) | GAD5 | F 80% | Age 15-80 y (median 70 y) | Neurocognitive disorders, LE, TLE with RRE, SPS, PERM, CA, DM1 (if titer < 20 U/ml) Frequent coexisting autonomic disturbances | Normal | -LE or mesiotemporal brain stem, cerebellum and spiral cord T2/FLAIR abnormalities evolving into atrophy. | Normal | None | Seizures > psychosis occurrence (age dependent) EEE: Rarely extreme delta train |

F: female; M: male; y: years; m: months; <: less than 20 cases reported; IM: immunotherapy; NMD: default mode network; LE: limbic encephalitis; GS: generalized slowing; FS: focal slowing; FIED: focal interictal epileptic discharges; FED: GEN: generalized epileptic discharges; OI: ovarian teratoma; SCLC: small cell lung carcinoma; ADC: adeno carcinoma; MD: hematological malignancies; HL: Hodgkin Lymphoma; NET: neuroendocrine tumor; LEMS: Lambert Eaton myasthenic syndrome; RE: rasmussens's; R: refractory status epilepticus; RS: refractory seizures; RFS: refractory focal seizures; TLE: temporal lobe epilepsy; SydCh: Sydenham chorea; TS: Tourette's syndrome; GDR: global developmental regression; AI: autonomic instability; MD: movement disorders; Cho: chorea; My: myoclonus; Tr: tremor; MT: motor tics; CA: cerebellar ataxia; SPS: Stiff-person syndrome; PERM: progressive encephalomyelitis with rigidity and myoclonus; DM1: diabetes mellitus type 1; OSAS: obstruction sleep apnea syndrome.
Glutamate-N-methyl-D-aspartate receptor encephalitis is the most frequent form of NSAS in children.\textsuperscript{[11-14]} According to up-to-date researches it is also the most common pediatric form of encephalitis, with the only exception of acute demyelinating encephalomyelitis.\textsuperscript{[15,16]} With reference to other pediatric NSAS, reports are mostly anecdotal, with the only exception of the forms associated with voltage-gated potassium channel complex (VGKC) antibodies.\textsuperscript{[17-20]} [Table 1]

In this paper the authors propose a diagnostic pathway based both on literature and the experience that may help to obtain accurate identification of pediatric NSAS, with the aim to start an adequate and early treatment, and achieve a better clinical outcome.

**DIAGNOSTIC CLUES**

When a healthy child presents with unexpected symptoms such as seizures, sudden behavioral changes and movement disorders, causes like infections and traumas must be ruled out, together with toxic, metabolic and neoplastic factors. Another issue to be excluded is a previous central nervous system disease history. Once left aside all this, an autoimmune etiology should be always taken into account.

Longitudinal clinical, neurophysiological and neuroradiological findings facilitate the diagnostic pathway, and often provide information suggestive of specific NSAS variants [Figure 1].

Moreover, abnormalities at electroencephalogram (EEG) and magnetic resonance imaging (MRI) may be
roughly specific for the different phases of the disease, allowing monitoring its progression.

As reported in adults, the different forms of pediatric NSAS share an overlap of clinical signs and symptoms. Typically, onset is acute/subacute (< 12 weeks) and occurs simultaneously or after a stressful event, such as infections, fever or cephalalgia. Behavioral and sleep disorders, confusion and short term memory impairment are often present, but epileptic seizures are generally the first obvious sign in children, consisting in focal seizures arising from bi-temporal lobes or, less frequently, generalized ones. In this phase EEG recordings usually show a diffuse background activity slowness, sometimes associated with superimposed paroxysmal abnormalities, and MRI findings are usually normal or non specific.

Usually, a more acute stage follows, characterized by reduced consciousness, refractory/super-refractory epileptics status and a progressive sinking into a coma lasting days to weeks. Ventilator and/or other vital parameters support are often required, as well as a heavy sedation that might easily hide eventual associated symptoms.

In this phase, interictal EEG recordings may show a diffuse high delta waves activity with focal discharges, refractory multifocal status epilepticus or less frequently, extreme delta-brush with superimposed periodic lateralized epileptiform discharges. A close EEG monitoring is mandatory, inclusive of at least one 24 h video-EEG recording (duration to increase according to the clinical picture). On MRI, in this stage common findings are a T2/FLAIR hyperintensity in the medial temporal lobes involving the hippocampus, and/or the prefrontal areas and the cingulate gyrus. Only few exceptions are detectable, for example a frequent persistent negative findings in dipeptidyl-peptidase-like protein-6 encephalitis, an extensive multifoal or widespread diffuse abnormalities in NMDAR and GABAA-R encephalitis, and a basal ganglia involvement in D2-R encephalitis.

A slowly progressive improvement of the interictal activity usually follows, sometimes preceded by a previous sleep background activity reorganization (personal observation), which generally gives way to a drug-resistant epilepsy although, in a minority of cases, a complete recovery is achieved.
According to Suleiman classification, in order to help in the diagnosis 5 different autoimmune epilepsy categories are identifiable, on the basis both of the data obtained and of the immunotherapy response. Treatment may lead to remission, but the response depends on the specific form and possible association with malignancy.\textsuperscript{[7,45]} In some forms, such as NMDA-R encephalitis, a complete recovery occurs in about 80\% with a very low mortality rate.\textsuperscript{[13,14,46-48]} When a complete remission does not occur, sequelae ranging from drug-resistant epilepsy associated with cognitive decline, to milder cognitive impairment are easily detectable.\textsuperscript{[1,12,13,47]}

Following the treatment phase, EEG interictal activities slowly improve and longitudinal MRI studies can reveal a trend toward complete resolution, especially in some specific NSAS and when a rapid immunotherapy is administered. A more severe course towards a global atrophy predominantly affecting hippocampus, frontal and parietal regions usually characterizes the remaining cases.\textsuperscript{[43]} An EEG monitoring including awakeness and sleep recordings (e.g. 40 min cad) as well as a neuroradiological surveillance are thereby suggested.

Ideally, CSF and autoimmune responses should be investigated as soon as there is suspicion of autoimmune encephalitis.

**CSF FINDINGS**

As in adult, CSF usually shows a mild to moderate lymphocytic pleocytosis (< 100 white blood cells/μL), increased protein concentration (< 150 mg/dL), normal glucose level and frequently elevated IgG index; oligoclonal bands result detectable in about 50\% of cases.

In children, elevated CSF neopterin can be used as an additional marker of CNS inflammation.\textsuperscript{[10,49]}

A recent study underlies the role of the CXCL13 chemokine as a potential CSF biomarker of clinical outcome in anti-NMDAR encephalitis, its prolonged or secondary elevation suggesting a limited response to immunotherapy, an higher risk of relapses, and thereby the need to a more aggressive therapeutic approach.\textsuperscript{[50]}

**IMMUNOLOGICAL FINDINGS**

As soon as an autoimmune condition is suspected (at prodromal or early acute stage) and before immunotherapy is started, a serum and/or CSF sample must be taken for autoantibody testing, and a Ig total level as well as a study of lymphocytic profile should be performed, in order to get a value to compare later on with [Figure 2].

If NSAbs are detected, the diagnosis is usually easily achieved. On a practical level, the expanding NSAbs spectrum could make it difficult to choose which antibody to check first. In order to direct the diagnostic pathway, indirect immunohistochemistry on rat brain tissue or immunocytochemistry in primary rat neuronal culture can highlight staining patterns evocative for surface (e.g. neuropil) or intracellular antigens.

This preliminary screening must be confirmed by more specific techniques, such as cell-based assay, ELISA or radioimmunoassay.\textsuperscript{[51]} A blended approach reduces the false positive rate,\textsuperscript{[52]} providing an efficient diagnostic tool for pediatric NSAS.

A paraneoplastic cause is much less probable in children, so that testing for onconeural antibodies (Hu, Ma2, CV2/CRMP5, Ri, amphiphysin) can be not strictly necessary, at first instance at least. Nevertheless, if a paraneoplastic clinical picture shows-up, a commercial immunoblotting assay specifically designed can be properly used to manage a differential diagnosis.

**TUMOR SURVEILLANCE**

Because of the low prevalence of malignancy in pediatric NSAS, many authors consider tumor surveillance not strictly necessary in the first instance, with the only exception related to NMDA-R encephalitis. Nevertheless it may become increasingly relevant in patients who are older at the time of clinical onset.\textsuperscript{[13]}

**TREATMENT**

Due to antibodies pathogenicity, treatment is focused on reducing the serum antibodies titer. There is no consensus on the immunotherapy approach to carry out, but it has become increasingly clear that starting treatment as early as possible is crucial to achieve a better clinical outcome.\textsuperscript{[12,48]}

At seizure onset, antiepileptic therapy, with few exceptions, usually results ineffective.\textsuperscript{[9]}

During the acute phase, as the diagnostic work out may take time, starting immunotherapy empirically is highly recommended. Although some patients
undergo a complete recovery spontaneously, this is not frequent and it is not possible to identify the patients with a favorable outcome. Taking time before treatment waiting for immunological results or tumoral screening in anti-NMDAR cases is not recommended, not only because of the severity of the clinical findings but also because patients not promptly treated may be at higher risk for relapse.[12,53]

To date no consensus has been achieved on the treatment scheme to be used, and the available protocols are heterogeneous.

The first-line therapy usually includes a short course of high-dose steroids (methylprednisolone MP; 30 mg/kg/day i.v. per 3-5 days) followed by or combined with intravenous immunoglobulin (IvIg) administration (0.4 g/kg/day per 5 days).

Steroids are then tapered using 1-2 mg/kg/day orally, on average for another 12 weeks, adjusting the dose according to patient tolerability or possible side effects. If no benefit is noticed during steroid treatment, plasma exchange (PE), 3-5 cycles, should be considered.

In case the first-line treatment is unsatisfactory, a second-line immunotherapy should be started. It usually consists of rituximab 375 mg/mq per week every other week for 4 weeks,[54,55] cyclophosphamide (Cyc) 750 mg/m², 3 times or mycophenolate mophetil 600 mg/m², alone or in combination.[1,13]

The immunotherapy’s effectiveness can be checked with HIC on frozen rat brain tissue to assess the lack of immunostaining.

In the meanwhile, antiepileptic treatment is usually continued, even though its real impact in modifying the epileptic course remains uncertain as long as the immune mechanism starts to decrease itself. The decision whether to withdraw antiepileptic drugs or not should be made according to the patient, the specific disorder and EEG findings in the follow-up.

Psychiatric symptoms and involuntary movements, when present, can be treated symptomatically, and medications with a broad effect on multiple symptoms are usually recommended. Long acting benzodiazepines, sedatives such as clonidine, and anticonvulsivant drugs may be helpful in improving abnormal movements and mood instability. The management of psychiatric symptoms is more challenging; sedative and sleep medications other than benzodiazepines seem to be the most effective, while antipsychotic drugs are less efficacious and often associated with adverse events.[56]

Finally, during the remission and stabilization phases a gammaglobuline total check should be repeated in order to detect rituximab induced hypogammaglobulinemia, that can eventually be treated with a replacement of an extra dose of exogenous IvIg.[10,57]

Regarding relapse risk prevention, no data are available so far on the preventive value of chronic long term IvIg administration, but encouraging results come from the chance of monitoring the CD19+ and CD27+ lymphocytes value every 2 months, re-administering rituximab in case of their further increase.[58]

**DISCUSSION**

The early recognition of an immune mechanism underlying a neurologic disorder provides a chance to start early treatment and to achieve a better outcome.

Guidelines for NSAS in children have been recently developed,[10] extrapolated from a previous study by Zuliani[1] referred to adults, and mainly differing from it since focused on the higher epilepsy occurrence among pediatric symptoms.[10,59] As in Zuliani’s, the role given to immunotherapy response becomes a retrospective feature that helps with the classification itself. This points out that, whenever a specific antibody is detected, the diagnosis of NSAS is easily achieved; conversely, the hypothesis lies in a shady area. In this paper, based on a review of the literature and the experience, the authors provide a simplified pathway that may facilitate the identification and the early treatment of these forms. Concerning the diagnostic algorithm many questions remain unanswered.

A field that requires further work is the differential diagnosis among the individual forms of NSAS but this was beyond the aim of the paper. The spectrum of signs and symptoms is wide and it is often difficult to achieve a specific diagnosis on clinical ground only because of the overlapping of clinical signs. The recognition of some highly characteristic clinical features is sometimes possible and further work using an integrated approach combining EEG, neuroimaging and early identification of the underlying immunological mechanism is highly recommendable, as it can lead to an early appropriate treatment and to the possibility of a perceivable clinical improvement.
Few reports are available to date about the predictive value of electrophysiological findings in NSAS. Identifying specific EEG pathological patterns may help not only in distinguishing the different forms, but also in recognizing them at an initial stage, with the aim of administering an early treatment. Moreover MRI-patterns, despite providing a supportive feature in the diagnostic flow-chart orientation, are often quite specific, and thereby usually insufficient, if alone, to get specific NSAS distinguishable from each other, especially at clinical onset.

Correlations between electrophysiological and neuroradiological data are then mandatory, but remain partly unexplored. Modern neuroradiological techniques are now increasingly available and could be useful to better understand pathophysiological mechanism and disclose predictive outcome data in different NSAS.

Moreover, no consensus is obtained to date about the sensibility and specificity of serum vs. CSF testing. Although a study performed by Gresa-Arribas et al. in 2014 demonstrated the higher CSF testing reliability in NMDA-R encephalitis, consensual data lack on the others NSAS. Moreover, the same work stressed out a positive correlation between the antibody titer and the risk of relapse, but no predictive threshold value has been established to help in deciding to resort to a retreatment or a chronic immunotherapy.

Finally, concerning the therapeutic approach, strategies tailored to the individual syndrome should be outlined, considering a less aggressive approach for those with a usually better outcome, such as NMDA-R encephalitis. This must be done by taking into account the possible side effects of immunotherapeutic drugs and a “risks vs. benefits” assessment per single patient.

CONCLUSION

In children NSAS clinical picture is heterogeneous, often overlapping and still poorly outlined. Anyhow, the recognition of some characteristic clinical features are sometimes possible, and can help the diagnostic approach with the aim to start a proper and early treatment. Nevertheless, further studies on larger prospective pediatric cohorts and randomized treatment trials are required in order to assess the need to tailor more or less aggressive treatments according to long term outcome findings and prognostic factors in different NSAS.

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Conflicts of interest
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