Neonatal Seizures – are we there yet?

Journal:	*Neuropediatrics*
Manuscript ID	Draft
Manuscript Type:	Review Articles
Date Submitted by the Author:	n/a
Complete List of Authors:	Ramantani, Georgia; University Children's Hospital Zürich, Neuropediatrics Schmitt, Bernhard; University Children's Hospital, Neuropediatrics Plecko, Barbara; Univ. Children's Hospital, Medical University Graz Pressler, Ronit; Great Ormond Street Hospital For Children NHS Foundation Trust, Neurophysiology Wohlrab, Gabriele ; University Children´s Hospital Zurich, Neuropediatrics Klebermass-Schrehof, Katrin; Medical University of Vienna, Neonatology Hagmann, Cornelia; University Children's Hospital Zurich, Neonatology and Pediatric Intensive Care Pisani, Francesco; University-Hospital of Parma, Child Neuropsychiatry Unit Boylan, Geraldine; University College Cork, Paediatrics and Child Health
Keyword:	neonatal seizures, outcome, preterm infants, term infants
Please select the subject area of your manuscript::	Epilepsy
Abstract:	Neonatal seizures are the most prevalent and distinctive sign of neurologic dysfunction in early life and pose an immense challenge for clinicians. Improvements in neonatal care have increased the survival rate of extremely premature infants, considerably changing the spectrum of underlying etiologies, and instigating a gradual shift from mortality to morbidity. Recognizing neonatal seizures can be challenging due to variability in presentation, but clinical features can often provide valuable clues about etiology. Even though conventional EEG with simultaneous video-detection of ictal events still represents the diagnostic gold standard, continuous monitoring using a 1-2 channel amplitude-integrated EEG with simultaneous unprocessed EEG can be crucial for early recognition and intervention. Furthermore, tremendous progress has been made in neuroimaging. While the majority of neonatal seizures are caused by hypoxic-ischemic events, stroke, hemorrhage or infection, about 15% of patients will require more sophisticated algorithms for diagnostic workup, including metabolic and genetic screening. These recent developments have led to renewed interest in the classification of neonatal seizures, which aim to help identify etiology and guide appropriate therapeutic and prognostic decisions. In this review, we outline recent progress made in the etiology, diagnosis and treatment of neonatal seizures and highlight areas that deserve further research.

Neonatal Seizures – are we there yet?

Georgia Ramantani[1], Bernhard Schmitt[1], Barbara Plecko[1,2], Ronit M Pressler[3,4], Gabriele Wohlrab[1], Katrin Klebermass-Schrehof[5], Cornelia Hagmann[6], Francesco Pisani[7], Geraldine B. Boylan[8,9]

[1]Department of Neuropediatrics, University Children's Hospital, Zurich, Switzerland; [2]Department of Pediatrics and Adolescent Medicine, Medical University Graz, Graz, Austria; [3]Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK; [4]UCL Great Ormond Street Institute for Child Health, London, UK; [5]Division of Neonatology, Pediatric Intensive Care and Neuropediatrics, Department of Pediatrics, Medical University Vienna, Vienna, Austria; [6]Department of Neonatology and Pediatric Intensive Care, University Children's Hospital, Zurich, Switzerland; [7]Child Neuropsychiatry Unit, University-Hospital of Parma, Parma, Italy; [8]Irish Centre for Fetal and Neonatal Translational Research (INFANT), University College Cork, Ireland; [9]Department of Pediatrics and Child Health, University College Cork, Cork, Ireland.

Correspondence to: Georgia Ramantani, MD, PhD; Department of Neuropediatrics, University Children's Hospital Zurich, Steinwiesstrasse 75, 8032 Zurich, Switzerland *E-mail*: georgia.ramantani@kispi.uzh.ch

Word count: 4425; **number of references:** 130; **number of tables:** 1; **number of figures**: 6

ABSTRACT

Neonatal seizures are the most prevalent and distinctive sign of neurologic dysfunction in early life and pose an immense challenge for clinicians. Improvements in neonatal care have increased the survival rate of extremely premature infants, considerably changing the spectrum of underlying etiologies, and instigating a gradual shift from mortality to morbidity. Recognizing neonatal seizures can be challenging due to variability in presentation, but clinical features can often provide valuable clues about etiology. Even though conventional EEG with simultaneous video-detection of ictal events still represents the diagnostic gold standard, continuous monitoring using a 1-2 channel amplitude-integrated EEG with simultaneous unprocessed EEG can be crucial for early recognition and intervention. Furthermore, tremendous progress has been made in neuroimaging and all infants with seizures should have an MRI to help identify the underlying etiology. While the majority of neonatal seizures are caused by hypoxic-ischemic events, stroke, hemorrhage or infection, about 15% of patients will require more sophisticated algorithms for diagnostic workup, including metabolic and genetic screening. These recent developments have led to renewed interest in the classification of neonatal seizures, which aim to help identify etiology and guide appropriate therapeutic and prognostic decisions. In this review, we outline recent progress made in the etiology, diagnosis and treatment of neonatal seizures and highlight areas that deserve further research.

Keywords: neonatal seizures, outcome, preterm, term infants

INTRODUCTION

Seizure incidence is higher during the neonatal period than at any other time of life [1]. Neonatal seizures are the most common neurological emergency and are associated with a high risk of mortality and morbidity [2–4]. Neonatal seizures occur in 1-3 per 1000 live births [5–8], with substantially higher rates reported in preterm infants [9]. Improvements in neonatal care over the last few decades have changed the spectrum of injury seen in the immature brain and have facilitated a decrease in mortality following neonatal seizures. However, the prevalence of long-term morbidity in survivors remains unchanged [10,11].

Neonatal seizures are unique, as the majority are symptomatic of brain injury occurring acutely in the perinatal period; in stark contrast to seizures presenting later in infancy and childhood. Hypoxic-ischemic encephalopathy (HIE) in term neonates and intraventricular hemorrhage (IVH) in preterm infants are the most prevalent etiology in neonates. Other common causes are cerebral infarction, central nervous system (CNS) infection, brain malformation, or metabolic disorders [11].

In the past decade, tremendous progress has been made in the area of neonatal seizure detection and etiological classification using continuous neuro-monitoring and cutting-edge neuroimaging, in addition to clinical observation. Challenges in diagnostics have been met with the development of metabolic as well as genetic screening, which carry the potential for rapid diagnosis and novel treatment options. In spite of increasing awareness about neonatal seizures and their dire consequences, including the high prevalence of cerebral palsy, developmental delay and post-neonatal epilepsy, little progress has been made in the development of effective treatments for neonatal seizures. Randomized controlled trials have never been more urgent.

In this review, we highlight key areas of neonatal seizure diagnosis and treatment, and identify the most imperative questions that still remain unanswered.

Classification of neonatal seizures

Neonatal seizures are usually acute symptomatic, often electrographic only (subclinical) or show discreet clinical manifestations that can be difficult to differentiate from movements seen in sick preterm or term babies [12,13]. Hence, the need for EEG confirmation of neonatal seizures is widely accepted [6,12]. However, this hinders the integration of neonatal seizures into a classification scheme serving all ages, which is reflected by the fact that, until recently, the ILAE seizure classification did not include neonatal seizures [14,15]. It is not surprising therefore that other classifications have been published by neonatologists and pediatric neurologists, which are unique to the neonatal period [13,16]. However, these were based on clinical semiology only [16], neglected electrographic seizures [16], and included epileptic and non-epileptic events [13,16].

In 2014, a new Task Force on Neonatal Seizures was established by the ILAE (International League Against Epilepsy - Commission for terminology and classification). This Task force has developed a diagnostic framework based on the Mizrahi classification of neonatal seizures and the 2017 ILAE seizure classification [17,18], which consists of four domains: clinical presentation (high risk or clinical suspicious events), diagnosis (with EEG), manifestation (with or without clinical manifestation) and seizure types (motor: automatisms, clonic, epileptic spasms,, myoclonic, sequential and tonic and non-motor: autonomic and behavioural arrest, as well as unclassified). This classification has several advantages: it is tailored towards neonates and ignores seizure types not seen in this age group, it includes electrographic seizures and allows the user to choose the degree of detail when classifying seizures:

https://www.ilae.org/guidelines/definition-and-classification/neonatal-seizure-classification. At the same time the same terminology and seizure types as in the 2017 ILAE classification are used: https://www.ilae.org/journals/epigraph/epigraph-vol-17-issue-3-fall-2015/classifying-seizures-in-the-very-young-initial-plans-of-the-neonatal-seizure-task-force-part-of-ilae-commission-on-classification-and-terminology.

Does seizure semiology reveal seizure etiology?

Seizure phenomena in neonates differ from those observed in older infants, and this is particularly the case for premature neonates [1]. Recognizing seizures in the neonatal period can be challenging due to variability in presentation [19,20], and clinical suspicion should be verified by EEG recording, where possible, before treatment initiation. Although amplitude-integrated EEG (aEEG) is a particularly useful tool in the neonatal intensive care unit (NICU), full video-EEG remains the gold standard for the detection of epileptic seizures in neonates.

A wide range of underlying causes give rise to seizures in neonates, including HIE, intracranial hemorrhage or infarction, metabolic or electrolyte disorders, CNS infections or congenital malformations, and genetic disorders. Neonatal epileptic syndromes [14] such as self-limiting familial neonatal epilepsy (BFNE), early myoclonic encephalopathy (EME), and Early infantile epileptic encephalopathy (Ohtahara syndrome) are infrequent. Despite the inherent complexity in this long list of causes and variable seizure semiologies, clinical features of neonatal seizures can suggest the underlying etiology and help guide appropriate treatment options.

True myoclonic seizures should raise suspicions of a metabolic disorder such as non-ketotic hyperglycinemia (NKH), propionic acidemia, and vitamin B_6-dependent epilepsy. A variety of severe brain insults, e.g., cortical malformations as well as metabolic and genetic disorders (*PEX*, *ARX*, *CDKL5*, *KCNQ2*, *SPTAN*, *STXBP1*-related epilepsy etc.) are associated with tonic

seizures. Epileptic spasms in neonates are rare, mostly found in metabolic disorders, but can also be caused by cortical malformations or early epileptic encephalopathy. Sequential seizures with spasms are suggestive of a vitamin B_6 dependent epilepsy [22,23]. Nearly all seizure types have been reported in neonates with HIE but a large proportion will be electrographic-only [13,21]. Focal clonic seizures point to a focal cortical lesion (stroke, intracranial hemorrhage, focal cortical dysplasia) [13]. Sequential seizures, encompassing a sequence of a tonic followed by a myoclonic or clonic phase have been observed in neonates with *KCNQ2*-mutations and a variable severity of clinical presentation [24].

HIE accounts for 60-65% of neonatal seizures occurring in the first day of life and most cases are evidenced by a complicated birth history. Neonatal seizures occurring up to 72 hours after birth may be associated with stroke or brain malformations, bacterial meningitis, intrauterine infection, IVH in preterm neonates, drug withdrawal, and metabolic-genetic disorders, whereas those occurring towards the end of the first week of life in otherwise healthy neonates with a family history of neonatal seizures may point to self-limiting familial neonatal epilepsy [23,25–27]. The affected infants present with a normal developmental trajectory with seizures gradually stopping by 6 months of age [25]. Mutations in two potassium channel subunit genes are associated with self-limiting familial neonatal epilepsy. Potassium voltage-gated channel subfamily KQT member 2 (KCNQ2) mutations are the most common, whereas KCNQ3 mutations are rare [25,28,29]. Another autosomal dominant epilepsy syndrome presenting with neonatal seizures is the self-limiting familial neonatal-infantile epilepsy, associated with mutations in the sodium channel subunit gene *SCN2A* [30,31]. Seizure onset in this disorder varies and seizures can start in the neonatal or infantile period and generally stop by 12 months of age [25].

Overall, the variability of seizure types and the extensive list of etiologies pose a tremendous challenge to the diagnostic skills of even the most experienced clinicians. Yet, clinical features in neonatal seizures have the potential to help reveal the underlying etiology and thus facilitate the implementation of a suitable treatment.

Ictal and interictal EEG

An electrographic seizure is a sudden, abnormal EEG event defined by a repetitive and evolving pattern with a voltage of >2μV and a duration of >10sec [32]. "Evolving" is defined as an unequivocal evolution in frequency, voltage, morphology, or location. An interval of at least 10sec is required to separate two distinct seizures [32]. Nevertheless, cut-offs are arbitrary and exceptions to the rule may occur. For example, epileptic generalized myoclonic jerks are associated with discharges of <10sec duration. Brief rhythmic discharges of <10sec duration without clinical symptoms are considered non-ictal, although they can have the same characteristics and bear the same risk for mortality and neurologic disability as electrographic seizures [33]. Other critical aspects are the demarcation of the onset and the end of the ictal discharge from interictal activity and the differentiation of seizures from seizure-like artefacts, physiological or pathological non-ictal rhythmic patterns or periodic discharges [34].

Electrographic seizures can be:

- *unifocal*: multiple seizures arise from a single region (Figs. 1, 2)
- *multifocal*: seizures originate from at least three independent foci with at least one in each hemisphere
- *lateralized*: seizures propagate within a single hemisphere
- *bilateral independent*: seizures occur simultaneously in two regions and begin, evolve, and behave independently

- *bilateral:* involvement of both hemispheres (Fig. 3)
- *migrating*: the seizure moves sequentially from one hemisphere to another, or
- *diffuse*: asynchronous involvement of all brain regions

The morphology of ictal discharges consists of rhythmic spikes, sharp-waves or rhythmic beta-, alpha-, theta- or delta waves. In preterm infants, rhythmic delta waves are the most common ictal pattern [35]. Focal clonic or focal tonic seizures exhibit focal EEG discharges, while generalized myoclonic jerks are associated with generalized bursts [13]. Ictal EEGs are often focal in origin [36], while not necessarily corresponding to an underlying focal pathology.

Status epilepticus is diagnosed when the summed duration of seizures comprises ≥50% of an arbitrarily defined one-hour epoch [32]. So-called periodic patterns are of uncertain significance. These are described as relatively uniform patterns with waveforms recurring at almost regular intervals without evolution, lasting >10sec, presenting different morphologies, and focal, bilateral synchronous, bilateral asynchronous or diffuse localizations [32].

A normal background pattern in an infant with unremarkable neurological examination and motor seizures may suggest self-limiting familial neonatal epilepsy [25,26]. Seizures in self-limiting familial neonatal epilepsy, as recently characterized in a large cohort, may be focal or generalized clonic or tonic, often associated with apnea, head or eye deviation, or staring [25].

An interictal burst-suppression pattern is a characteristic pattern of early onset epileptic encephalopathy with onset in the first month of life i.e. Ohtahara syndrome, or early infantile epileptic encephalopathy and early myoclonic encephalopathy [37–40]. Tonic seizures are the predominant seizure type in Ohtahara syndrome, whereas myoclonic seizures are the predominant seizure type in early myoclonic encephalopathy. Ohtahara syndrome and early myoclonic encephalopathy were recently considered part of a spectrum, with a considerable

overlap in clinical presentation and etiology [41]. Known genetic causes of Ohtahara syndrome and early myoclonic encephalopathy include brain malformations (e.g., polymicrogyria and lissencephaly), inborn errors of metabolism (e.g., pyridoxine- and other vitamin-dependent epilepsies, mitochondrial disorders, and amino acidopathies), and other genetic etiologies (e.g., pathogenic variants in ARX, GABRA1, KCNQ2, KCNT1 SCN2A, SIK1, SLC25A22, STXBP1) [24,42–48]. Overall, single gene variants underlie > 20-30% of epileptic encephalopathies [49–51]. The identification of these genetic etiologies may prove crucial for patients with early-onset refractory epilepsy who may profit from gene-based treatments in light of emerging precision medicine [52].

aEEG in seizure monitoring

While full video-EEG remains the gold standard for neurophysiological monitoring, amplitude-integrated EEG (aEEG), which displays a time-compressed, one-or two-channel trend of the EEG, is increasingly utilized for long-term monitoring and continuous surveillance in the NICU. This simplified monitoring enables the assessment of the background activity and facilitates the earlier recognition of state changes, but abnormal findings (especially suspected seizure activity) require further investigation by more detailed full EEG.

Full EEG is, however, difficult to implement on a 24/7 basis in non-expert centres. aEEG on the other hand provides much needed information when continuous full EEG monitoring is not available. This is especially the case for infants with neonatal seizures, when the aEEG can help assess seizure burden and the impact of anticonvulsive therapy. Previous literature has shown an 80% correlation of seizure detection by aEEG compared to full EEG [53] when used by aEEG experts, showing that aEEG-based seizure diagnosis is much more reliable than clinical diagnosis alone [54,55]. When non-experts assessed the aEEG results were, however, much

poorer [56]. Seizures are more common over central cerebral regions and, if EEG electrodes cover this area, neonatal seizures can be identified in 70-80% of cases [57]. Seizures can be detected in the aEEG as 'saw-tooth-like' augmentations of the baseline amplitude but should be confirmed by examination of the simultaneous raw-EEG trace to rule out any artefact (Fig. 4). Thus, aEEG can facilitate the verification of 'clinical seizure' diagnosis, detect subclinical seizures, monitor the effect of anticonvulsants and is a useful aid for clinical decision making in the NICU, particularly when full EEG monitoring is either not feasible or not available. However, it should be borne in mind that short-term, focal, and low-amplitude seizures may be missed [57–59].

Seizure treatment studies that compared clinical diagnosis alone with aEEG-based continuous monitoring for seizure detection showed a lower injury score on MRI and a lower epilepsy incidence later in life when aEEG monitoring was available [60,61]. The reduction of total seizure burden by optimized aEEG-guided treatment correlated with improved cognitive outcome in neonates suffering from hypoxic-ischemic encephalopathy [62]. In conclusion, continuous simplified monitoring of cerebral function by aEEG has the potential to support the diagnosis and treatment of neonatal seizures, particularly in non-specialist centres.

Neuroimaging of neonatal seizures

Neuroimaging techniques used in neonatal seizures include cranial ultrasound (cUS) and MRI. Although most NICUs use cUS as the method of choice, MRI is rapidly gaining ground with the majority of neonates with seizures or HIE in recent studies undergoing at least one MRI scan [63,64]. The distinct advantages of cUS are the wider availability, the feasibility of bedside use in all infants including those too unstable to be transported to the MRI unit, and its compatibility with minimal handling in very immature neonates. However, the acquisition of high quality cUS

images is user-dependent, thus posing clear limitations for the detection of certain brain injuries. On the other hand, MRI is not always available and requires a transfer of the neonate to a dedicated MRI unit. Nevertheless, MRI has been acknowledged as the optimal neuroimaging modality for neonatal seizures, particularly when age-appropriate acquisition protocols are applied [65]. Ultimately a combination of these two techniques could provide the ideal tools to evaluate the underlying etiology.

The added value of MRI compared to cUS has been assessed in a large cohort of term and near-term infants with different seizure etiologies [66]. In all but 6% of infants, the underlying etiology could be identified, helped significantly by MRI [66]. In 12% of infants, a diagnosis or major imaging abnormalities would have been missed if only cUS rather than a combination of cUS and MRI had been used. As expected, MRI was most useful in diagnosing cerebral sinus venous thrombosis, some metabolic disorders and cerebral dysgenesis [66]. Another study showed that the probability of neurodevelopmental impairment or recurrent seizures was low in the absence of significant cerebral lesions on MRI [67], highlighting the utility of MRI not only in identifying the cause of neonatal seizures but also in providing information on long-term outcome.

Magnetic resonance spectroscopy (MRS) can contribute information additional to conventional MRI in the evaluation of neonatal seizures by noninvasively measuring central nervous system metabolite levels such as N-acetylaspartate (NAA), choline, creatine, and lactate. Abnormal lactate, pyruvate or amino acid peaks may point to inborn errors of metabolism [68] and MRS may guide the detection of mitochondrial disease in neonates with normal MRI [69]. Furthermore, MRS has the potential to contribute information relevant to prognosis in HIE [70]. Several studies have shown that lactate/creatine plus phosphocreatine, lactate/NAA, or lactate/choline–containing compounds peak-area ratios in HIE provide accurate prognostic

markers of the severity of brain injury and subsequent neurodevelopmental outcome, before changes are apparent on conventional MRI [71–75].

Measuring the efficacy of neonatal seizure treatment

To date, few studies have used a standardized protocol for measuring seizure treatment efficacy in neonates. Many older studies relied on the clinical abolition of seizures only as a measure of treatment efficacy: this is clearly not adequate. aEEG efficacy measurement is better, but there are some limitations already outlined that make aEEG inadequate for use in randomized controlled trials. Full EEG has been used in a number of small studies to measure treatment efficacy, but the methods used were heterogeneous; information on the length of time it took for seizures to reduce or abate was rarely included, and the percentage change in seizures from baseline was not discussed. This makes a comparison between studies particularly challenging and a meta-analysis almost impossible. As a result, it has been difficult to progress studies of anti-epileptic drug (AED) treatment in neonates. Measuring treatment outcomes for neonatal seizures can also be difficult because of the natural history of neonatal seizures, and this can vary with etiology [76].

We advocate the use of multichannel video EEG monitoring during neonatal seizure trials and that the accumulated duration of electrographic seizures, often referred to as seizure burden, should be the quantitative measure of choice when assessing AED efficacy [77–80]. Seizure burden can be measured in minutes per hour and is a measure of the short-term intensity of seizures. Video-EEG technology has advanced dramatically in the last ten years, and recordings can be stored either locally or centrally. Most importantly, it is now possible to review live cot-side video-EEG recordings remotely, making EEG more accessible and allowing for 24-hour interpretation. Seizure detection algorithms are currently undergoing randomized trials, and there

is no doubt that this technology will very soon make it easier to automatically calculate the on-going seizure burden and evolving seizure profile [81,82].

It has long been recognized that neonatal seizures evolve over time but very few studies have detailed the evolution of electrographic seizures in neonates and those that have, generally describe seizures in neonates with HIE [76,83–85]. Lynch et al. examined the temporal distribution of seizures in neonates with HIE and found that seizures had a short period of high electrographic seizure burden near the time of seizure onset, followed by a longer period of low seizure burden [76].

Neonatal seizure evolution does not only depend on etiology, and factors such as gestational age and treatment are also important (Fig. 5). However, it is not known if earlier treatment of electrographic seizures will alter the course of the seizure evolution and result in less brain injury though some studies do indicate that a lower seizure burden is associated with less severe MRI severity scores and better outcomes [60,62,86]. Due to logistic challenges in EEG monitoring and recruitment [12], studies that aim to treat electrographic seizures immediately after onset are rare [60,62,79].

Metabolic and genetic work up in pharmacoresistant neonatal seizures

Whilst most neonatal seizures are symptomatic and usually related to HIE (38%), ischemic stroke (18%), and intracranial hemorrhage (11%) [6], a subgroup of about 13% represent distinct neonatal epilepsy syndromes, related to either brain malformations or genetic aetiologies [87]. Within this subgroup, congenital brain malformations, detectable by neuroimaging, have been established in 41% of neonatal epilepsies in a recent study, whereas genetic aetiologies were identified in 42% [87], with an overlap of about 9% between structural and genetic causes. Inborn errors of metabolism, established on the grounds of clinical presentation and biochemical

investigations -and often verified by genetic work up- represent a major challenge that needs to be identified –and addressed- quickly in order to avoid metabolic decompensation and enable counselling regarding recurrence risks and overall prognosis [88,89].

As early diagnosis enables specific treatment in some metabolic disorders [90] and may influence the choice of drugs in primary genetic conditions, a diagnostic algorithm should be in place in all neonatal units. This should include a standardized and well-documented vitamin B_6 trial (Fig. 6), which may identify patients with defects in *ALDH7A1* [91], *PNPO* [92], the newly described *PLPBP (*previously named *PROSC) gene* [93,94] or rare cases of severe congenital hypophosphatasia [95]. These patients manifest with myoclonic seizures or a variety of other seizure types that are typically resistant to standard anticonvulsants and may be associated with a burst suppression pattern in EEG. Respective biomarkers can be used to guide further diagnostic workup of inborn errors of metabolism (Table 1).

Patients with molybdenum cofactor deficiency (MocD) manifest with tonic clonic seizures, poor feeding, and variable facial dysmorphic signs. In this disorder, neuroimaging is quite specific, with findings ranging from cerebral oedema to cystic leukoencephalopathy [96]. For MocD type A, substitution with purified cyclic pyranopterin monophosphate cPMP has proven effective, but the window of opportunity is very short [97]. The past decade has revealed a quickly growing number of genes that cause primary genetic early onset epileptic encephalopathies [98]. Some may have suggestive semiology, such as unilateral tonic and prolonged seizures in KCNQ2 mutations, while in, e.g., STXBP1 mutations, broad phenotypic variability has been described [99]. Thus, many institutions have changed their policies by sequencing multiple genes in a panel approach or going for next generation sequencing of the whole exome [89] with a diagnostic yield of about 40% in patients with seizure onset < 2 months of age [100]. As

mutations in some genes occur de novo, while others are of Mendelian inheritance, an exact diagnosis is crucial for further family planning and counselling.

The need for trials in neonatal seizures

Considering that a high seizure burden may aggravate long-term outcome, there is an urgent need to control prolonged or recurrent seizures. Nevertheless, there is still an open debate concerning the management of neonatal seizures [101]. As a first step, the underlying etiology of seizures must be established as soon as possible, since this can facilitate an etiological and effective treatment. As a second step, for symptomatic treatment, a short-term or long-term therapy should be chosen, depending on the risk of seizure recurrence.

One of the major issues in the management of neonatal seizures is the lack of effective antiepileptic drugs. In a Cochrane review from 2004 [102], only two randomized controlled trials could be identified, with the authors concluding that "there is little evidence from randomized controlled trials to support the use of any of the anticonvulsants currently used in the neonatal period." Phenobarbital, the most widely-used first-line drug in neonatal seizures, has a response rate of approximately 43% and phenytoin, as second-line AED, of 57% [77]. Benzodiazepines and levetiracetam are commonly used as second or third-line drugs. Lidocaine reached a response rate of 68% in full-term neonates with a higher response rate than midazolam as second-line AED (p = 0.049) [103]. However, lidocaine toxicity, mainly in the form of cardiac arrhythmias, can be life threatening [104,105]. Furthermore, in view of potential cardiac side effects, lidocaine cannot be combined with phenytoin [106]. However in a recent study involving 368 full-term and 153 preterm infants, lidocaine-associated cardiac events were rare, especially since the introduction of new reduced-dose regimens [107]. Furthermore, no specific

AED treatments for preterm infants are indicated, in spite of the vast differences in pharmacokinetics as well as in the maturation of the CNS.

To address this, the NEMO (NEonatal Seizure Using Medication Off-patent) consortium set out in 2009 to evaluate the loop diuretic bumetanide as a potential second line treatment for neonatal seizures in a multi-centre study across Europe. This study was, unfortunately, stopped early because of possible ototoxicity concerns and limited evidence for seizure reduction. In the past decade, several AEDs, such as levetiracetam [108,109] and topiramate [110], have emerged as viable alternatives with the potential to address age-specific mechanisms and challenges, but randomized controlled trials are still pending [101].

Finally, it is still unclear if improved control of neonatal seizures has the potential to enhance long-term outcome and this will remain an open issue until effective treatments are found. New generation AEDs appear promising, considering the absence of pro-apoptotic properties [101,111], but there is still an urgent need for randomized controlled studies in neonates.

Outcome of neonatal seizures

Mortality following neonatal seizures has decreased from 40% to 20% in the last few decades. However the prevalence of long-term neurological sequelae in survivors remains unchanged at 30% [1,11]. The incidence of postneonatal epilepsy, cerebral palsy and developmental delay is higher in preterm neonates [8,112], with a reported odds ratio of 14 (95% CI, 2-86) per week of gestational age [113]. This shift from mortality to morbidity in the preterms poses a significant challenge for clinical management in the NICU [114]. In a recent study [115], unfavourable outcome predictors in preterm neonates included low birth-weight, low Apgar score at 1 minute, abnormalities in neurologic examination, pathologic EEG or cUS findings, and particularly neonatal status epilepticus (a rarity at low gestational ages).

Moreover, recent preclinical [116] and clinical [117,118] studies in HIE have provided evidence that recurrent seizures themselves may amplify injury to the developing brain beyond that of the underlying etiology. Overall, experimental data support the belief that seizures in early life impede normal development and reduce the efficiency of cortical networks, even in the absence of cell loss [119,120]. Permanent impairments in learning, memory, and cognition, as well as increased seizure susceptibility, may result from these seizure-induced changes in neuronal connectivity and receptor expression [121,122]. Interestingly, animal models provide evidence that prolonged seizures or status epilepticus result in brain injury only in the presence of pre-existing insults such as those associated with HIE [123]. These observations are crucial in terms of neonatal seizure management, but experimental data still awaits confirmation in prospective double blind clinical studies. It should be noted that a 2016 Cochrane review investigating prophylactic barbiturate use in HIE [124] reported a reduced risk of seizures but no reduction in neonate mortality, whereas long-term outcomes were unavailable.

In the meantime, several – usually single centre – studies have sought to identify outcome predictors, mainly in the underlying etiology or specific seizure types and EEG patterns [10]. Research on this topic is, however, impeded by the variable criteria of neonatal seizure identification and etiologic diagnosis throughout research studies [10,114], with preterm neonates constituting a particular challenge in this respect. Nevertheless, considerable efforts have been made to develop a robust scoring system/predictive model for neonatal seizures that would facilitate clinical decision [125–129]. These models are yet to be validated in larger, representative contemporary cohorts, to promote their implementation in clinical practice.

The increased availability of continuous video-EEG and/or aEEG monitoring in diagnosis and treatment evaluation of neonatal seizures is offering more refined diagnostic and therapeutic approaches. Furthermore, biomarkers such as semiology and EEG are expected to play a new

role in the context of genetic disease [87], and novel therapies [108,109] deriving from lab research and aiming to minimize damage to the immature brain [130] are expected to improve long-term outcomes. Predictive models and scoring systems will have to adapt to this rapidly changing landscape of neonatal seizures and their outcomes.

CONCLUSION

Recent technological advances in diagnostics, including full EEG, aEEG, MRI, metabolic and genetic testing, have improved seizure detection and etiologic classification in neonates. Meanwhile, ground breaking preclinical research on the effects of seizures and AEDs in the immature brain has improved our understanding of this complex situation. However, little has changed in terms of treatment and, consequently, the long-term outcomes, with neonatal seizures continuing to pose a challenge for clinicians worldwide. Research must continue to facilitate the decoding of the mechanisms underlying neonatal seizures, improve their management by developing age-specific agents, and, ultimately, improving long-term outcomes in affected infants.

CONFLICTS OF INTEREST

The authors have no conflict of interest to disclose. This work did not receive support from a grant or otherwise. Part of this work was presented at the Symposium *Neonatal seizures – update*, in Zurich, Switzerland, 24.06.2016.

REFERENCES

1. Volpe JJ. Neurology of the Newborn. 5th Edition. Philadelphia: Elsevier Ltd, Oxford; 2008.
2. Pisani F, Cerminara C, Fusco C, Sisti L. Neonatal status epilepticus vs recurrent neonatal seizures: clinical findings and outcome. Neurology 2007;69:2177–85.

3. Pisani F, Barilli AL, Sisti L, Bevilacqua G, Seri S. Preterm infants with video-EEG confirmed seizures: outcome at 30 months of age. Brain Dev. 2008;30:20–30.

4. Berry K, Pesko MF, Hesdorffer DC, Shellhaas RA, Seirup JK, Grinspan ZM. An evaluation of national birth certificate data for neonatal seizure epidemiology. Epilepsia 2017;58:446–55.

5. Lanska MJ, Lanska DJ, Baumann RJ, Kryscio RJ. A population-based study of neonatal seizures in Fayette County, Kentucky. Neurology 1995;45:724–32.

6. Glass HC, Shellhaas RA, Wusthoff CJ, et al. Contemporary Profile of Seizures in Neonates: A Prospective Cohort Study. J. Pediatr. 2016;

7. Saliba RM, Annegers FJ, Waller DK, Tyson JE, Mizrahi EM. Risk factors for neonatal seizures: a population-based study, Harris County, Texas, 1992-1994. Am. J. Epidemiol. 2001;154:14–20.

8. Ronen GM, Buckley D, Penney S, Streiner DL. Long-term prognosis in children with neonatal seizures: a population-based study. Neurology 2007;69:1816–22.

9. Kohelet D, Shochat R, Lusky A, Reichman B, Israel Neonatal Network. Risk factors for neonatal seizures in very low birthweight infants: population-based survey. J. Child Neurol. 2004;19:123–8.

10. Ramantani G. Neonatal epilepsy and underlying aetiology: to what extent do seizures and EEG abnormalities influence outcome? Epileptic Disord. 2013;15:365–75.

11. Silverstein FS, Jensen FE. Neonatal seizures. Ann. Neurol. 2007;62:112–20.

12. Boylan GB, Stevenson NJ, Vanhatalo S. Monitoring neonatal seizures. Semin. Fetal. Neonatal Med. 2013;18:202–8.

13. Mizrahi EM, Kellaway P. Characterization and classification of neonatal seizures. Neurology 1987;37:1837–44.

14. Berg AT, Berkovic SF, Brodie MJ, et al. Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE Commission on Classification and Terminology, 2005-2009. Epilepsia 2010;51:676–85.

15. Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 1981;22:489–501.

16. Volpe JJ. Neonatal seizures: current concepts and revised classification. Pediatrics 1989;84:422–8.

17. Fisher RS, Cross JH, French JA, et al. Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology. Epilepsia 2017; 58:522-530.

18. Fisher RS, Cross JH, D'Souza C, et al. Instruction manual for the ILAE 2017 operational classification of seizure types. Epilepsia 2017;58:531–42.

19. Sivaswamy L. Approach to neonatal seizures. Clin. Pediatr. (Phila.) 2012;51:415–25.

20. Nagarajan L, Palumbo L, Ghosh S. Classification of clinical semiology in epileptic seizures in neonates. Eur. J. Paediatr. Neurol. 2012;16:118–25.

21. Mizrahi EM, Kellaway P. Diagnosis and management of neonatal seizures. Philadelphia: Lippincott-Raven Publishers; 1998.

22. Schmitt B, Baumgartner M, Mills PB, et al. Seizures and paroxysmal events: symptoms pointing to the diagnosis of pyridoxine-dependent epilepsy and pyridoxine phosphate oxidase deficiency. Dev. Med. Child Neurol. 2010;52:e133-142.

23. Ronen GM, Rosales TO, Connolly M, Anderson VE, Leppert M. Seizure characteristics in chromosome 20 benign familial neonatal convulsions. Neurology 1993;43:1355–60.

24. Weckhuysen S, Ivanovic V, Hendrickx R, Van Coster R, Hjalgrim H, Møller RS, et al. Extending the KCNQ2 encephalopathy spectrum: clinical and neuroimaging findings in 17 patients. Neurology 2013;81:1697–703.

25. Grinton BE, Heron SE, Pelekanos JT, et al. Familial neonatal seizures in 36 families: Clinical and genetic features correlate with outcome. Epilepsia 2015;56:1071–80.

26. Watanabe K. Seizures in the newborn and young infants. Folia Psychiatr. Neurol. Jpn. 1981;35:275–80.

27. Van Hove JLK, Lohr NJ. Metabolic and monogenic causes of seizures in neonates and young infants. Mol. Genet. Metab. 2011;104:214–30.

28. Zara F, Specchio N, Striano P, et al. Genetic testing in benign familial epilepsies of the first year of life: clinical and diagnostic significance. Epilepsia 2013;54:425–36.

29. Soldovieri MV, Boutry-Kryza N, Milh M, et al. Novel KCNQ2 and KCNQ3 mutations in a large cohort of families with benign neonatal epilepsy: first evidence for an altered channel regulation by syntaxin-1A. Hum. Mutat. 2014;35:356–67.

30. Concolino D, Iembo MA, Rossi E, et al. Familial pericentric inversion of chromosome 5 in a family with benign neonatal convulsions. J. Med. Genet. 2002;39:214–6.

31. Vigevano F. Benign familial infantile seizures. Brain Dev. 2005;27:172–7.

32. Tsuchida TN, Wusthoff CJ, Shellhaas RA, et al. American clinical neurophysiology society standardized EEG terminology and categorization for the description of continuous EEG monitoring in neonates: report of the American Clinical Neurophysiology Society critical care monitoring committee. J. Clin. Neurophysiol. 2013;30:161–73.

33. Nagarajan L, Palumbo L, Ghosh S. Brief electroencephalography rhythmic discharges (BERDs) in the neonate with seizures: their significance and prognostic implications. J. Child Neurol. 2011;26:1529–33.

34. Shewmon DA. What is a neonatal seizure? Problems in definition and quantification for investigative and clinical purposes. J. Clin. Neurophysiol. 1990;7:315–68.

35. Fenichel GM. Neonatal Neurology. 4th ed. London, United Kingdom: Churchill Livingstone; 2007.

36. Scher MS, Hamid MY, Steppe DA, Beggarly ME, Painter MJ. Ictal and interictal electrographic seizure durations in preterm and term neonates. Epilepsia 1993;34:284–8.

37. Ohtahara S, Yamatogi Y. Ohtahara syndrome: with special reference to its developmental aspects for differentiating from early myoclonic encephalopathy. Epilepsy Res. 2006;70 Suppl 1:S58-67.

38. Djukic A, Lado FA, Shinnar S, Moshé SL. Are early myoclonic encephalopathy (EME) and the Ohtahara syndrome (EIEE) independent of each other? Epilepsy Res. 2006;70 Suppl 1:S68-76.

39. Yamatogi Y, Ohtahara S. Early-infantile epileptic encephalopathy with suppression-bursts, Ohtahara syndrome; its overview referring to our 16 cases. Brain Dev. 2002;24:13–23.

40. Beal JC, Cherian K, Moshe SL. Early-onset epileptic encephalopathies: Ohtahara syndrome and early myoclonic encephalopathy. Pediatr. Neurol. 2012;47:317–23.

41. Olson HE, Kelly M, LaCoursiere CM, et al. Genetics and genotype-phenotype correlations in early onset epileptic encephalopathy with burst suppression. Ann. Neurol. 2017;81:419–29.

42. Nakamura K, Kato M, Osaka H, et al. Clinical spectrum of SCN2A mutations expanding to Ohtahara syndrome. Neurology 2013;81:992–8.

43. Pavone P, Spalice A, Polizzi A, Parisi P, Ruggieri M. Ohtahara syndrome with emphasis on recent genetic discovery. Brain Dev. 2012;34:459–68.

44. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nat. Genet. 2008;40:782–8.

45. Saitsu H, Kato M, Okada I, et al. STXBP1 mutations in early infantile epileptic encephalopathy with suppression-burst pattern. Epilepsia 2010;51:2397–405.

46. Marques I, Sá MJ, Soares G, et al. Unraveling the pathogenesis of ARX polyalanine tract variants using a clinical and molecular interfacing approach. Mol. Genet. Genomic Med. 2015;3:203–14.

47. Kodera H, Ohba C, Kato M, et al. De novo GABRA1 mutations in Ohtahara and West syndromes. Epilepsia 2016;57:566–73.

48. Ohba C, Kato M, Takahashi N, et al. De novo KCNT1 mutations in early-onset epileptic encephalopathy. Epilepsia 2015;56:e121-128.

49. Allen NM, Conroy J, Shahwan A, et al. Unexplained early onset epileptic encephalopathy: Exome screening and phenotype expansion. Epilepsia 2016;57:e12-17.

50. Epi4K Consortium, Epilepsy Phenome/Genome Project, Allen AS, Berkovic SF, Cossette P, Delanty N, et al. De novo mutations in epileptic encephalopathies. Nature 2013;501:217–21.

51. Veeramah KR, Johnstone L, Karafet TM, et al. Exome sequencing reveals new causal mutations in children with epileptic encephalopathies. Epilepsia 2013;54:1270–81.

52. EpiPM Consortium. A roadmap for precision medicine in the epilepsies. Lancet Neurol. 2015;14:1219–28.

53. Toet MC, van der Meij W, de Vries LS, Uiterwaal CSPM, van Huffelen KC. Comparison between simultaneously recorded amplitude integrated electroencephalogram (cerebral function monitor) and standard electroencephalogram in neonates. Pediatrics 2002;109:772–9.

54. Murray DM, Boylan GB, Ali I, Ryan CA, Murphy BP, Connolly S. Defining the gap between electrographic seizure burden, clinical expression and staff recognition of neonatal seizures. Arch. Dis. Child. Fetal Neonatal Ed. 2008;93:F187-191.

55. Frenkel N, Friger M, Meledin I, et al. Neonatal seizure recognition--comparative study of continuous-amplitude integrated EEG versus short conventional EEG recordings. Clin. Neurophysiol. 2011;122:1091–7.

56. Rennie JM, Chorley G, Boylan GB, Pressler R, Nguyen Y, Hooper R. Non-expert use of the cerebral function monitor for neonatal seizure detection. Arch. Dis. Child. Fetal Neonatal Ed. 2004;89:F37-40.

57. Shellhaas RA, Soaita AI, Clancy RR. Sensitivity of amplitude-integrated electroencephalography for neonatal seizure detection. Pediatrics 2007;120:770–7.

58. Shah DK, Mackay MT, Lavery S, et al. Accuracy of bedside electroencephalographic monitoring in comparison with simultaneous continuous conventional electroencephalography for seizure detection in term infants. Pediatrics 2008;121:1146–54.

59. Shellhaas RA, Barks AK. Impact of amplitude-integrated electroencephalograms on clinical care for neonates with seizures. Pediatr. Neurol. 2012;46:32–5.

60. van Rooij LGM, Toet MC, van Huffelen AC, et al. Effect of treatment of subclinical neonatal seizures detected with aEEG: randomized, controlled trial. Pediatrics 2010;125:e358-366.

61. Toet MC, Groenendaal F, Osredkar D, van Huffelen AC, de Vries LS. Postneonatal epilepsy following amplitude-integrated EEG-detected neonatal seizures. Pediatr. Neurol. 2005;32:241–7.

62. Srinivasakumar P, Zempel J, Trivedi S, et al. Treating EEG Seizures in Hypoxic Ischemic Encephalopathy: A Randomized Controlled Trial. Pediatrics 2015;136:e1302-1309.

63. Tekgul H, Gauvreau K, Soul J, et al. The current etiologic profile and neurodevelopmental outcome of seizures in term newborn infants. Pediatrics 2006;117:1270–80.

64. Barnette AR, Horbar JD, Soll RF, et al. Neuroimaging in the evaluation of neonatal encephalopathy. Pediatrics 2014;133:e1508-1517.

65. Weeke LC, Van Rooij LGM, Toet MC, Groenendaal F, de Vries LS. Neuroimaging in neonatal seizures. Epileptic Disord. 2015;17:1–11; quiz 11.

66. Weeke LC, Groenendaal F, Toet MC, et al. The aetiology of neonatal seizures and the diagnostic contribution of neonatal cerebral magnetic resonance imaging. Dev. Med. Child Neurol. 2015;57:248–56.

67. Osmond E, Billetop A, Jary S, Likeman M, Thoresen M, Luyt K. Neonatal seizures: magnetic resonance imaging adds value in the diagnosis and prediction of neurodisability. Acta Paediatr. 1992 2014;103:820–6.

68. Zand DJ, Simon EM, Pulitzer SB, et al. In vivo pyruvate detected by MR spectroscopy in neonatal pyruvate dehydrogenase deficiency. AJNR 2003;24:1471–4.

69. Bianchi MC, Tosetti M, Battini R, et al. Proton MR spectroscopy of mitochondrial diseases: analysis of brain metabolic abnormalities and their possible diagnostic relevance. AJNR 2003;24:1958–66.

70. Rincon SP, Blitstein MBK, Caruso PA, González RG, Thibert RL, Ratai E-M. The Use of Magnetic Resonance Spectroscopy in the Evaluation of Pediatric Patients With Seizures. Pediatr. Neurol. 2016;58:57–66.

71. Barkovich AJ, Baranski K, Vigneron D, et al. Proton MR spectroscopy for the evaluation of brain injury in asphyxiated, term neonates. AJNR 1999;20:1399–405.

72. Amess PN, Penrice J, Wylezinska M, et al. Early brain proton magnetic resonance spectroscopy and neonatal neurology related to neurodevelopmental outcome at 1 year in term infants after presumed hypoxic-ischaemic brain injury. Dev. Med. Child Neurol. 1999;41:436–45.

73. Hanrahan JD, Cox IJ, Azzopardi D, et al. Relation between proton magnetic resonance spectroscopy within 18 hours of birth asphyxia and neurodevelopment at 1 year of age. Dev. Med. Child Neurol. 1999;41:76–82.

74. Cheong JLY, Cady EB, Penrice J, Wyatt JS, Cox IJ, Robertson NJ. Proton MR spectroscopy in neonates with perinatal cerebral hypoxic-ischemic injury: metabolite peak-area ratios, relaxation times, and absolute concentrations. AJNR 2006;27:1546–54.

75. Fan G, Wu Z, Chen L, Guo Q, Ye B, Mao J. Hypoxia-ischemic encephalopathy in full-term neonate: correlation proton MR spectroscopy with MR imaging. Eur. J. Radiol. 2003;45:91–8.

76. Lynch NE, Stevenson NJ, Livingstone V, Murphy BP, Rennie JM, Boylan GB. The temporal evolution of electrographic seizure burden in neonatal hypoxic ischemic encephalopathy. Epilepsia 2012;53:549–57.

77. Painter MJ, Scher MS, Stein AD, et al. Phenobarbital compared with phenytoin for the treatment of neonatal seizures. N. Engl. J. Med. 1999;341:485–9.

78. Boylan GB, Rennie JM, Pressler RM, Wilson G, Morton M, Binnie CD. Phenobarbitone, neonatal seizures, and video-EEG. Arch. Dis. Child. Fetal Neonatal Ed. 2002;86:F165-170.

79. Pressler RM, Boylan GB, Marlow N, et al. Bumetanide for the treatment of seizures in newborn babies with hypoxic ischaemic encephalopathy (NEMO): an open-label, dose finding, and feasibility phase 1/2 trial. Lancet Neurol. 2015;14:469–77.

80. Abend NS, Gutierrez-Colina AM, Monk HM, Dlugos DJ, Clancy RR. Levetiracetam for treatment of neonatal seizures. J. Child Neurol. 2011;26:465–70.

81. Mathieson SR, Stevenson NJ, Low E, et al. Validation of an automated seizure detection algorithm for term neonates. Clin. Neurophysiol. 2016;127:156–68.

82. Haas. Efficacy of Intravenous Levetiracetam in Neonatal Seizures (NEOLEV2) [Internet]. ClinicalTrials.gov: U.S. NAtional Institutes of Health; 2016. Available from: https://clinicaltrials.gov/ct2/show/NCT01720667.

83. Low E, Boylan GB, Mathieson SR, et al. Cooling and seizure burden in term neonates: an observational study. Arch. Dis. Child. Fetal Neonatal Ed. 2012;97:F267-272.

84. Wusthoff CJ, Dlugos DJ, Gutierrez-Colina A, et al. Electrographic seizures during therapeutic hypothermia for neonatal hypoxic-ischemic encephalopathy. J. Child Neurol. 2011;26:724–8.

85. Glass HC, Wusthoff CJ, Shellhaas RA, et al. Risk factors for EEG seizures in neonates treated with hypothermia: a multicenter cohort study. Neurology 2014;82:1239–44.

86. Kharoshankaya L, Stevenson NJ, Livingstone V, et al. Seizure burden and neurodevelopmental outcome in neonates with hypoxic-ischemic encephalopathy. Dev. Med. Child Neurol. 2016;58:1242–8.

87. Shellhaas RA, Wusthoff CJ, Tsuchida TN, et al. Profile of neonatal epilepsies: Characteristics of a prospective US cohort. Neurology 2017; 89:893-899.

88. Dulac O, Plecko B, Gataullina S, Wolf NI. Occasional seizures, epilepsy, and inborn errors of metabolism. Lancet Neurol. 2014;13:727–39.

89. Scheffer IE. Epilepsy genetics revolutionizes clinical practice. Neuropediatrics 2014;45:70–4.

90. Campistol J, Plecko B. Treatable newborn and infant seizures due to inborn errors of metabolism. Epileptic Disord. 2015;17:229–42.

91. Stockler S, Plecko B, Gospe SM, et al. Pyridoxine dependent epilepsy and antiquitin deficiency: clinical and molecular characteristics and recommendations for diagnosis, treatment and follow-up. Mol. Genet. Metab. 2011;104:48–60.

92. Mills PB, Camuzeaux SSM, Footitt EJ, et al. Epilepsy due to PNPO mutations: genotype, environment and treatment affect presentation and outcome. Brain 2014;137:1350–60.

93. Darin N, Reid E, Prunetti L, et al. Mutations in PROSC Disrupt Cellular Pyridoxal Phosphate Homeostasis and Cause Vitamin-B6-Dependent Epilepsy. Am. J. Hum. Genet. 2016;99:1325–37.

94. Plecko B, Zweier M, Begemann A, et al. Confirmation of mutations in PROSC as a novel cause of vitamin B 6 -dependent epilepsy. J. Med. Genet. 2017; 54:809-814.

95. Baumgartner-Sigl S, Haberlandt E, Mumm S, et al. Pyridoxine-responsive seizures as the first symptom of infantile hypophosphatasia caused by two novel missense mutations (c.677T>C, p.M226T; c.1112C>T, p.T371I) of the tissue-nonspecific alkaline phosphatase gene. Bone 2007;40:1655–61.

96. Appignani BA, Kaye EM, Wolpert SM. CT and MR appearance of the brain in two children with molybdenum cofactor deficiency. AJNR 1996;17:317–20.

97. Schwahn BC, Van Spronsen FJ, Belaidi AA, et al. Efficacy and safety of cyclic pyranopterin monophosphate substitution in severe molybdenum cofactor deficiency type A: a prospective cohort study. Lancet 2015;386:1955–63.

98. McTague A, Howell KB, Cross JH, Kurian MA, Scheffer IE. The genetic landscape of the epileptic encephalopathies of infancy and childhood. Lancet Neurol. 2016;15:304–16.

99. Stamberger H, Nikanorova M, Willemsen MH, et al. STXBP1 encephalopathy: A neurodevelopmental disorder including epilepsy. Neurology 2016;86:954–62.

100. Trump N, McTague A, Brittain H, et al. Improving diagnosis and broadening the phenotypes in early-onset seizure and severe developmental delay disorders through gene panel analysis. J. Med. Genet. 2016;53:310–7.

101. Pressler RM, Mangum B. Newly emerging therapies for neonatal seizures. Semin. Fetal. Neonatal Med. 2013;18:216–23.

102. Booth D, Evans DJ. Anticonvulsants for neonates with seizures. Cochrane Database Syst. Rev. 2004;CD004218.

103. Weeke LC, Toet MC, van Rooij LGM, et al. Lidocaine response rate in aEEG-confirmed neonatal seizures: Retrospective study of 413 full-term and preterm infants. Epilepsia 2016;57:233–42.

104. van den Broek MPH, Rademaker CMA, van Straaten HLM, et al. Anticonvulsant treatment of asphyxiated newborns under hypothermia with lidocaine: efficacy, safety and dosing. Arch. Dis. Child. Fetal Neonatal Ed. 2013;98:F341-345.

105. van den Broek MPH, Huitema ADR, van Hasselt JGC, et al. Lidocaine (lignocaine) dosing regimen based upon a population pharmacokinetic model for preterm and term neonates with seizures. Clin. Pharmacokinet. 2011;50:461–9.

106. Donovan MD, Griffin BT, Kharoshankaya L, Cryan JF, Boylan GB. Pharmacotherapy for Neonatal Seizures: Current Knowledge and Future Perspectives. Drugs 2016;76:647–61.

107. Weeke LC, Schalkwijk S, Toet MC, van Rooij LGM, de Vries LS, van den Broek MPH. Lidocaine-Associated Cardiac Events in Newborns with Seizures: Incidence, Symptoms and Contributing Factors. Neonatology 2015;108:130–6.

108. Ramantani G, Ikonomidou C, Walter B, Rating D, Dinger J. Levetiracetam: safety and efficacy in neonatal seizures. Eur. J. Paediatr. Neurol. 2011;15:1–7.

109. Fürwentsches A, Bussmann C, Ramantani G, Ebinger F, Philippi H, Pöschl J, et al. Levetiracetam in the treatment of neonatal seizures: a pilot study. Seizure 2010;19:185–9.

110. Glass HC, Poulin C, Shevell MI. Topiramate for the treatment of neonatal seizures. Pediatr. Neurol. 2011;44:439–42.

111. Ikonomidou C, Turski L. Antiepileptic drugs and brain development. Epilepsy Res. 2010;88:11–22.

112. Yıldız EP, Tatlı B, Ekici B, et al. Evaluation of etiologic and prognostic factors in neonatal convulsions. Pediatr. Neurol. 2012;47:186–92.

113. Shah DK, Zempel J, Barton T, Lukas K, Inder TE. Electrographic seizures in preterm infants during the first week of life are associated with cerebral injury. Pediatr. Res. 2010;67:102–6.

114. Pisani F, Spagnoli C. Neonatal Seizures: A Review of Outcomes and Outcome Predictors. Neuropediatrics 2015;

115. Pisani F, Facini C, Pelosi A, Mazzotta S, Spagnoli C, Pavlidis E. Neonatal seizures in preterm newborns: A predictive model for outcome. Eur. J. Paediatr. Neurol. 2016;20:243–51.

116. Nardou R, Ferrari DC, Ben-Ari Y. Mechanisms and effects of seizures in the immature brain. Semin. Fetal. Neonatal Med. 2013;18:175–84.

117. Glass HC, Glidden D, Jeremy RJ, Barkovich AJ, Ferriero DM, Miller SP. Clinical Neonatal Seizures are Independently Associated with Outcome in Infants at Risk for Hypoxic-Ischemic Brain Injury. J. Pediatr. 2009;155:318–23.

118. Miller SP, Weiss J, Barnwell A, et al. Seizure-associated brain injury in term newborns with perinatal asphyxia. Neurology 2002;58:542–8.

119. Holmes GL. Effects of seizures on brain development: lessons from the laboratory. Pediatr. Neurol. 2005;33:1–11.

120. Ben-Ari Y, Holmes GL. Effects of seizures on developmental processes in the immature brain. Lancet Neurol. 2006;5:1055–63.

121. Brooks-Kayal AR. Rearranging receptors. Epilepsia 2005;46 Suppl 7:29–38.

122. Lee CL, Hannay J, Hrachovy R, Rashid S, Antalffy B, Swann JW. Spatial learning deficits without hippocampal neuronal loss in a model of early-onset epilepsy. Neuroscience 2001;107:71–84.

123. Wirrell EC, Armstrong EA, Osman LD, Yager JY. Prolonged seizures exacerbate perinatal hypoxic-ischemic brain damage. Pediatr. Res. 2001;50:445–54.

124. Young L, Berg M, Soll R. Prophylactic barbiturate use for the prevention of morbidity and mortality following perinatal asphyxia. Cochrane Database Syst. Rev. 2016;CD001240.

125. Pisani F, Sisti L, Seri S. A scoring system for early prognostic assessment after neonatal seizures. Pediatrics 2009;124:e580-587.

126. Garfinkle J, Shevell MI. Prognostic factors and development of a scoring system for outcome of neonatal seizures in term infants. Eur. J. Paediatr. Neurol. EJPN Off. J. Eur. Paediatr. Neurol. Soc. 2011;15:222–9.

127. Anand V, Nair PMC. Neonatal seizures: Predictors of adverse outcome. J. Pediatr. Neurosci. 2014;9:97–9.

128. Lai Y-H, Ho C-S, Chiu N-C, Tseng C-F, Huang Y-L. Prognostic factors of developmental outcome in neonatal seizures in term infants. Pediatr. Neonatol. 2013;54:166–72.

129. Ellison PH, Largent JA, Bahr JP. A scoring system to predict outcome following neonatal seizures. J. Pediatr. 1981;99:455–9.

130. Galanopoulou AS, Moshé SL. Neonatal and Infantile Epilepsy: Acquired and Genetic Models. Cold Spring Harb. Perspect. Med. 2015;6:a022707.

FIGURE LEGENDS

Fig. 1: Term neonate age 2 days, hypoxic-ischemic encephalopathy, focal clonic seizures involving the left arm and the left leg. The EEG seizure starts with rhythmic alpha waves evolving into irregular sharp theta waves and after 15 sec (not shown) in rhythmic sharp waves.

Fig. 2: Term neonate age 1 day, hypoxic-ischemic encephalopathy, focal clonic seizures involving the left arm and oral automatisms. The EEG seizure starts with rhythmic delta waves.

Fig. 3: Term neonate age 10 days, *STXBP1* encephalopathy, bilateral clonic seizures involving both arms and legs. The EEG seizure starts with bilateral amplitude reduction followed by bilateral parasagittal and generalized rhythmic spike waves with centro-median maximum.

Figure 4. aEEG (above) and EEG traces (below) depicting a seizure pattern in a neonate.

Figure 5. Seizures in 2 neonates showing the evolving seizure burden. The red vertical lines indicate the administration of loading doses of Phenobarbitone and the green vertical lines represent the administration of loading doses of a second line anticonvulsant (Phenytoin or Midazolam). The neonate in A has a total seizure burden of 243mins with 185 seizures; the neonate in B has a total seizure burden of 214mins with 56 seizures. The middle black trace denotes the neurophysiologist annotation of seizures, and the bottom blue trace denotes the period of therapeutic hypothermia. Both neonates had periods of status epilepticus, i.e. seizure burden of >30 min/h. Reproduced from Boylan et al 2013 [12].

Figure 6: Proposed algorithm for a standardized vitamin B_6 trial. The timing and switch from pyridoxine HCL to pyridoxal 5´-phosphate (PLP) is individual and should be considered after 24h on pyridoxine in case of persistent high seizure frequency. Improvement on EEG can lag markedly behind clinical improvement and is thus not a basis for initial decision-making. The algorithm does not exclude the simultaneous use of conventional anticonvulsants.

TABLE 1

Disease	Urin	Plasma	CSF	Gene
Antiquitin deficiency	↑ AASA, ↑ PA	↑ PA	↑ AASA, P6C, ↓PLP, ↑ PA, sec NT abn.	*ALDH7A1*
PNPO deficiency	(Vanillactate)	B_6 profile ↑ pyridoxamine	↓ PLP, sec NT abnorm.	*PNPO*
Congenital Hypophosphatasia		↓ AP, B_6 profile ↑ PLP	(↓ PLP ?)	*TNSALP*
MOCOD, ISOD	sulfocysteine ↑ AASA, ↑ P6C	↓ uric acid	↑ AASA, P6C ↓PLP, ↑ PA	*MOCS1, MOCS2, GPNH*
NKH (non ketotic hyperglycinemia)		aminoacids (glycine)	aminoacids (glycine) CSF/plasma >0.004	4-enzyme cleavage system
Organoacidurias (e.g. D2HGA)	organic acid profile	aminoacids		…
CDG syndromes		Transferrin isoelectric focusing		Common in CDG type II
Zellweger Syndrome		VLCFA, PA, phytanic acid, pristanic acid		*PEX* genes 1-13
Adenylosuccinate lyase deficiency	purines			*ADSL*

Table 1: Common metabolic diseases associated with neonatal seizures, their metabolic and genetic biomarkers. Specific biomarkers in preferred material are set in black, while biomarkers in non-preferred material, inconsistent and/or secondary findings, are set in grey.

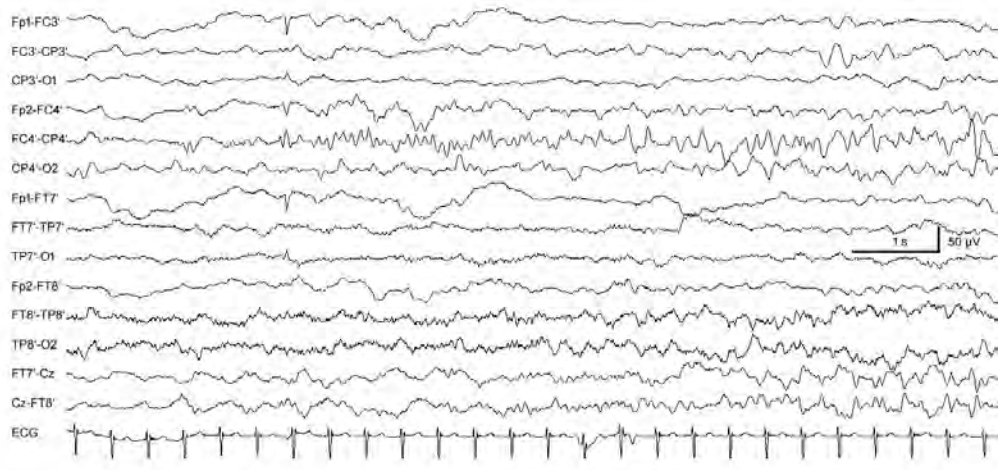

Fig. 1: Term neonate age 2 days, hypoxic-ischemic encephalopathy, focal clonic seizures involving the left arm and the left leg. The EEG seizure starts with rhythmic alpha waves evolving into irregular sharp theta waves and after 15 sec (not shown) in rhythmic sharp waves.

339x170mm (96 x 96 DPI)

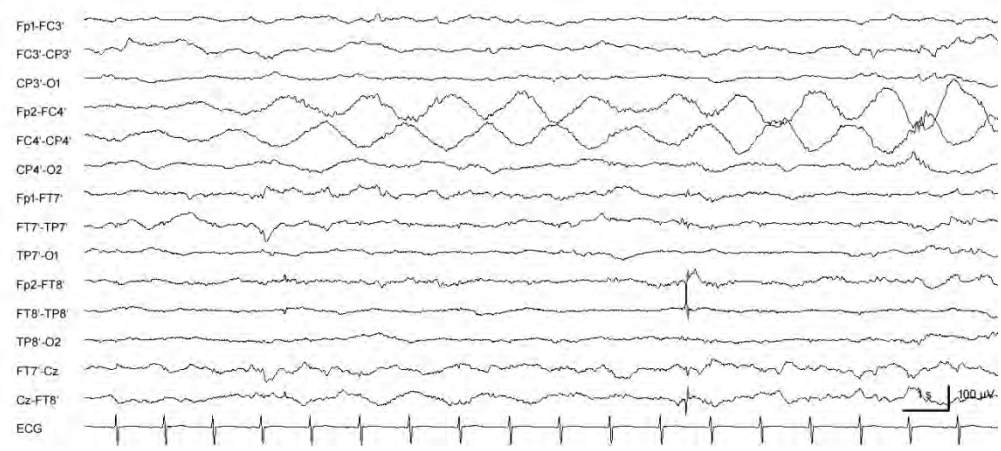

Fig. 2: Term neonate age 1 day, hypoxic-ischemic encephalopathy, focal clonic seizures involving the left arm and oral automatisms. The EEG seizure starts with rhythmic delta waves.

346x160mm (96 x 96 DPI)

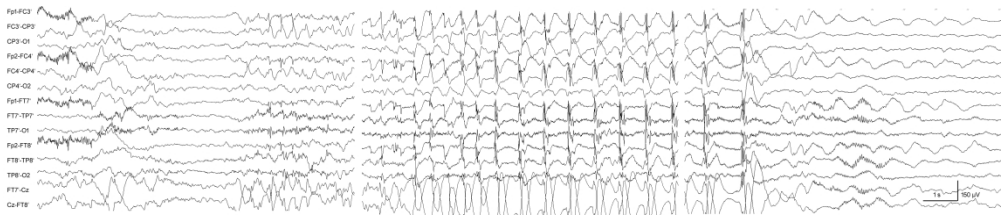

Fig. 3: Term neonate age 10 days, STXBP1 encephalopathy, bilateral clonic seizures involving both arms and legs. The EEG seizure starts with bilateral amplitude reduction followed by bilateral parasagittal and generalized rhythmic spike waves with centro-median maximum.

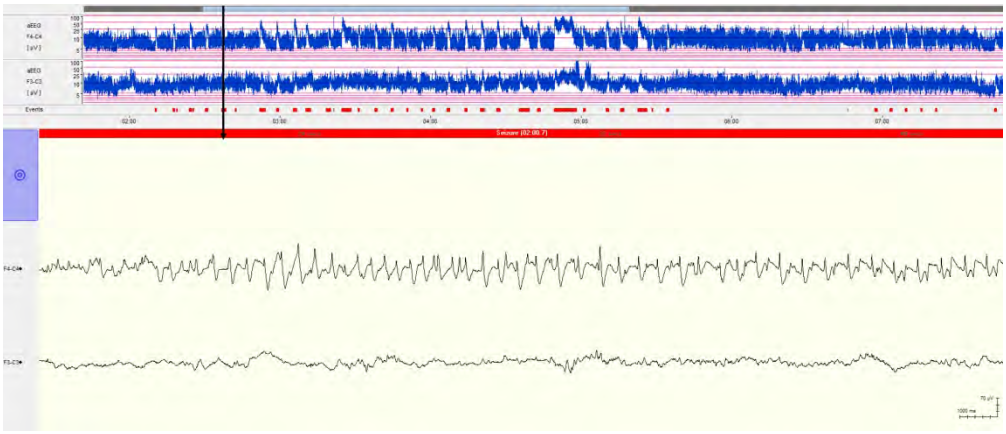

Figure 4. aEEG (above) and EEG traces (below) depicting a seizure pattern in a neonate.

329x140mm (150 x 150 DPI)

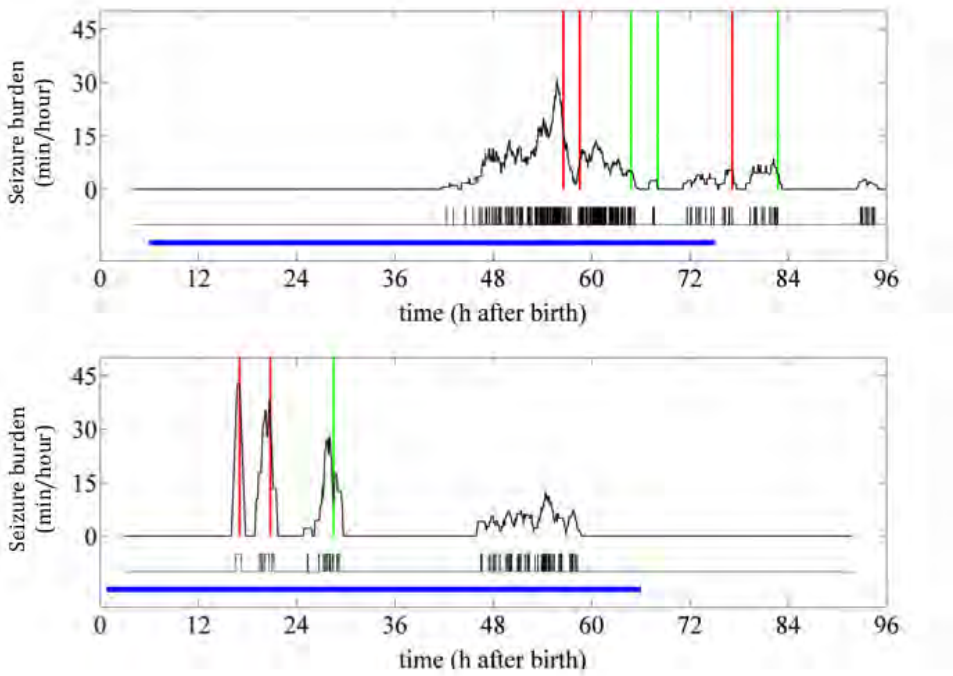

Figure 5. Seizures in 2 neonates showing the evolving seizure burden. The red vertical lines indicate the administration of loading doses of Phenobarbitone and the green vertical lines represent the administration of loading doses of a second line anticonvulsant (Phenytoin or Midazolam). The neonate in A has a total seizure burden of 243mins with 185 seizures; the neonate in B has a total seizure burden of 214mins with 56 seizures. The middle black trace denotes the neurophysiologist annotation of seizures, and the bottom blue trace denotes the period of therapeutic hypothermia. Both neonates had periods of status epilepticus, i.e. seizure burden of >30 min/h. Reproduced from Boylan et al 2013 [12].

254x190mm (72 x 72 DPI)

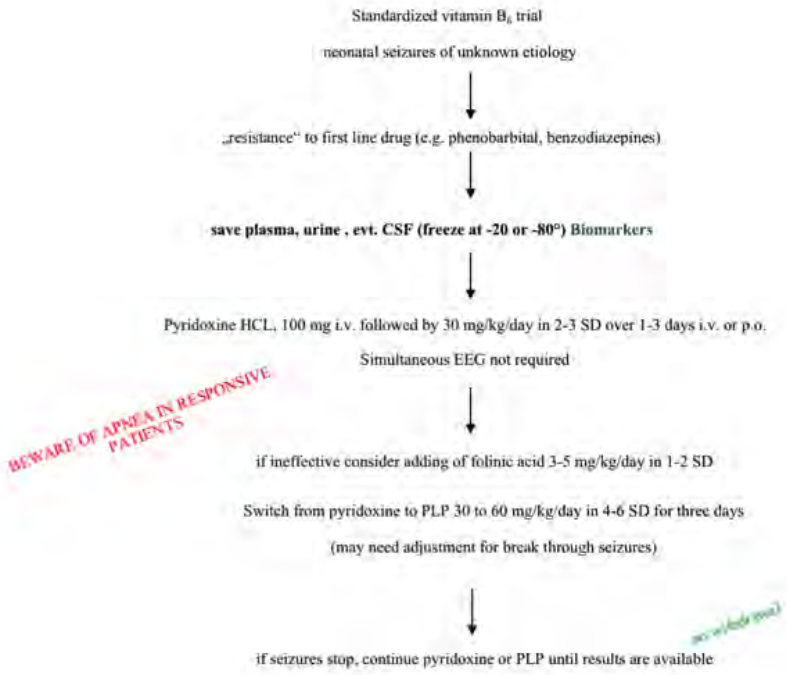

Figure 6: Proposed algorithm for a standardized vitamin B6 trial. The timing and switch from pyridoxine HCL to pyridoxal 5´-phosphate (PLP) is individual and should be considered after 24h on pyridoxine in case of persistent high seizure frequency. Improvement on EEG can lag markedly behind clinical improvement and is thus not a basis for initial decision-making. The algorithm does not exclude the simultaneous use of conventional anticonvulsants.

254x190mm (72 x 72 DPI)