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21 **Abstract**

22 Seizures are the most common neurological emergencies in the neonatal period and are  
23 associated with poor neurodevelopmental outcomes. Seizures affect up to five per 1000 term  
24 births and population based studies suggest that they occur even more frequently in premature  
25 infants. Seizures are a sign of an underlying cerebral pathology, the most common of which is  
26 hypoxic-ischemic encephalopathy in term infants. Due to a growing body of evidence that  
27 seizures exacerbate cerebral injury, effective diagnosis and treatment of neonatal seizures is of  
28 paramount importance to reduce long-term adverse outcomes. Electroencephalography is  
29 essential for the diagnosis of seizures in neonates due to their subtle clinical expression, non-  
30 specific neurological presentation and a high frequency of electro-clinical uncoupling in the  
31 neonatal period. Hypoxic-ischaemic encephalopathy may require neuroprotective therapeutic  
32 hypothermia, accompanying sedation with opioids, anticonvulsant drugs or a combination of  
33 all of these. The efficacy, safety, tolerability and pharmacokinetics of seven anticonvulsant  
34 drugs (phenobarbital, phenytoin, levetiracetam, lidocaine, midazolam, topiramate and  
35 bumetanide) are reviewed. This review is focused only on studies reporting electrographically  
36 confirmed seizures and highlights the knowledge gaps that exist in optimal treatment regimens  
37 for neonatal seizures. Randomised controlled trials are needed to establish a safe and effective  
38 treatment protocol for neonatal seizures.

39 *Key points:*

- 40 • The optimal treatment protocol for neonatal seizures remains elusive. Phenobarbital  
41 remains the first-line antiepileptic of choice, despite suboptimal efficacy and altered  
42 pharmacodynamic effects in neonates. There is currently no consensus regarding  
43 second-line drug choice, which often varies between phenytoin, lidocaine,  
44 levetiracetam or benzodiazepines.

- 45       • Hypothermia is the current standard of care for neuroprotection in HIE and many novel  
46       neuroprotective drugs are also emerging. Drug-drug interactions as well as drug-  
47       hypothermia interactions between antiepileptic drugs, novel neuroprotectants and  
48       hypothermia need to be investigated prior to administration in neonates, due to the  
49       potential for both pharmacokinetic and pharmacodynamic interactions.
- 50       • Continuous EEG monitoring is essential as a measure of antiepileptic efficacy.
- 51       • Randomised, controlled trials are required to establish a safe and effective treatment  
52       regimen for neonatal seizures.

### 53       **1. Neonatal seizures-an overview**

54       Neonatal seizures affect between one and five full-term neonates per 1000 live births, and are  
55       the most common neonatal neurological emergencies [1]. Moreover, the incidence of seizures  
56       increases in very low birth weight infants [2]. Phenobarbital remains the first line drug for  
57       treatment of neonatal seizures, despite having only around 50% efficacy [3]. There is little  
58       consensus about the best second-line treatment for neonatal seizures and there is considerable  
59       off-label use of antiepileptic medications with sparse efficacy data in the neonatal period.

#### 60       **1.1 Aetiology of neonatal seizures**

61       Seizures are a hallmark of neurological injury and approximately 60% of all neonatal seizures  
62       are attributable to hypoxic-ischaemic encephalopathy (HIE) [4]. In Europe, HIE is the third  
63       most common cause of neonatal mortality, accounting for 9% of all deaths and 21% of term  
64       deaths, while globally it is estimated to cause approximately one million neonatal deaths each  
65       year [5, 6]. For the survivors of HIE, there is significant secondary morbidity, including  
66       cerebral palsy (29%), cognitive delay (45%), seizure disorders (12%), sensorineural deafness  
67       (9%), and visual loss (26%) [7]. Seizures in HIE may exacerbate the underlying cerebral injury  
68       and increase the risk of detrimental neurodevelopmental consequences [8-11]. Perinatal arterial

69 ischaemic stroke is the second most common cause of seizures in term neonates and accounts  
70 for 7.5%-20% of neonatal seizures [12, 13]. Seizures also arise from intracranial haemorrhage  
71 (7%-18%), congenital cerebral malformations (3%-17%), infection (2%-14%), metabolic  
72 causes (3%-5%), electrolyte imbalances (1%-4%) and other less common causes [14, 15].  
73 There is little evidence on which to base recommendations for antiepileptic protocols  
74 regardless of seizure aetiology, although a prudent approach would be to treat the underlying  
75 cause and administer antiepileptic treatment according to hospital protocols.

## 76 **1.2 Pathophysiological aspects of neonatal seizures**

77 Seizures are the result of excessive electrical firing of neurons in the brain [16]. The immature  
78 brain is more susceptible to seizures, primed by the early development of excitatory  
79 neurotransmitters, delayed inhibitory function of gamma-amino butyric acid (GABA) and an  
80 excess of excitatory glutamatergic neurons which are composed of more excitable subunits  
81 than the equivalent in adults [17, 18].

82 The binding of GABA agonists/modulators to the GABA<sub>A</sub> receptor triggers either an influx or  
83 an efflux of chloride ions, depending on the neuronal equilibrium potential for chloride [19]. It  
84 has been shown that the expression of inward sodium-potassium-chloride cotransporter  
85 (NKCC1) in human cortex is increased at birth compared to one year of age, whereas  
86 expression of outward potassium-chloride cotransporter (KCC2) increases from birth onwards  
87 [17]. This leads to an accumulation of intracellular chloride in immature neurons through  
88 NKCC1, thus the equilibrium potential for chloride becomes positive in relation to the resting  
89 membrane potential [19]. In immature neurons, activation of the GABA<sub>A</sub> receptor results in  
90 chloride efflux and neuronal depolarisation [19]. Furthermore, birth injuries such as ischaemia  
91 increases NKCC1 and decrease KCC2 expression, whereas hypoxic-ischaemic injury increases  
92 NKCC1 alone [20]. For these reasons, treatment of neonatal seizures with GABA<sub>A</sub> agonists,  
93 such as phenobarbital or benzodiazepines, may be suboptimal. Moreover, GABA<sub>A</sub> receptors

94 are expressed at low levels in human and rodent cortex and contain less  $\alpha_1$  subunits than their  
95 adult counterparts, decreasing their sensitivity to modulation by benzodiazepines [21]. Female  
96 rats have increased levels of outward potassium-chloride transporter (KCC2), which translates  
97 to an inhibitory GABA action emerging earlier in females than in males [22]. Indeed, sex  
98 differences have been noted in mice, rats and humans with regards to susceptibility to brain  
99 injury, mechanisms of brain injury and response to treatment [23]. The increased seizure  
100 susceptibility due to developmental peculiarities of immature brain and excitatory GABA  
101 function might suggest that a class of antiepileptic drug (AED) other than GABA modulators  
102 should be considered as a first-line treatment for neonatal seizures. Furthermore, the sex  
103 differences observed in cotransporter expression raise questions regarding a differential  
104 approach to seizure treatment in male and female subjects.

### 105 **1.3 Diagnosis of neonatal seizures**

106 The diagnosis of neonatal seizures is challenging. Clinical seizure detection may lead to both  
107 over- and under-diagnosis [24]. Apart from classical tonic, clonic and myoclonic seizures,  
108 neonates may exhibit a wide variety of subtle seizure presentations including eye deviations,  
109 blinking, staring, chewing, sucking, cycling and boxing limb movements, apnoea and blood  
110 pressure changes [25]. Only a small portion of suspected neonatal clinical seizures are  
111 confirmed by electroencephalography (EEG) [24], while clinical signs may be absent in up to  
112 80-90% of electrographic seizures [26, 27]. The only randomised controlled trial comparing  
113 the effect of treatment of electrographic-only seizures to clinical-only seizures in neonates  
114 with HIE using a traditional AED protocol (phenobarbital up to 40 mg/kg, followed by  
115 fosphenytoin 20 mg/kg and third line midazolam bolus or infusion) demonstrated significantly  
116 reduced seizure burden in neonates treated based on electrographic seizure activity [28].

117 The absence or cessation of clinical correlates when electrographic seizures are confirmed is  
118 called electro-clinical uncoupling [29, 30]. The subtle clinical seizure presentation in neonates

119 and the phenomenon of electro-clinical uncoupling may be at least partly explained by the  
120 incomplete axonal dendritic and synaptic development, as well as incomplete myelination in  
121 the immature brain. Synaptic connectivity continues to increase until 2 years of age [31, 32].  
122 Clinical seizures can become even more difficult to detect following the administration of  
123 anticonvulsants or sedative agents, during hypothermia treatment or in neonates in critical  
124 condition [30, 33]. Both phenobarbital and phenytoin produced equal rates of uncoupling, with  
125 58% of neonates exhibiting only or mostly electrographic evidence of seizures after drug  
126 administration [30]. Differential maturation of transporters that control intracellular chloride  
127 levels in different regions of the brain could be the mechanism underlying AED-induced  
128 uncoupling. Phenobarbital, a GABA<sub>A</sub> agonist, reduced epileptiform power in slices taken from  
129 the ventroposterior thalamus of postnatal day 9/10 rat pups, but had no such effect on slices of  
130 neocortex from the same animals, suggesting that GABA signalling is inhibitory in the  
131 ventroposterior thalamus, but may be excitatory in the neocortex at this age [29].

132 A simplified and compressed version of multichannel EEG called amplitude-integrated EEG  
133 (aEEG) uses fewer channels than traditional EEG and requires less expertise for interpretation.  
134 It is often used for diagnosis of neonatal seizures in the neonatal intensive care unit (NICU)  
135 [1]. However, some seizures may be missed using this technology, as it struggles to identify  
136 low amplitude, short duration (< 1 minute) and infrequent seizures [27, 34, 35]. In addition,  
137 neonatal seizures often remain focal and do not generalise [27]. Therefore, focal seizures in the  
138 regions beyond aEEG electrode placement sites may remain undetected. Furthermore, artefacts  
139 that mimic seizure activity on aEEG may cause additional complications and lead to false  
140 positive readings [36]. Experience is required for reliable interpretation of both clinical and  
141 electrographic seizures, and studies have shown that non-expert users perform poorly in aEEG  
142 seizure detection [37]. There has been considerable effort in recent years to develop an  
143 automated neonatal seizure detection system to aid in clinical decision support in the NICU

144 and one such algorithm is currently undergoing a clinical trial across Europe (NCT02160171)  
145 [38, 39].

146 Reliable diagnosis of neonatal seizures can only be performed using continuous EEG (cEEG)  
147 monitoring which is considered the gold standard for the diagnosis of all neonatal seizures and  
148 for the assessment of anticonvulsant efficacy [24]. The role of cEEG monitoring extends to the  
149 differential diagnosis of seizure aetiology, particularly for HIE, stroke, infantile  
150 encephalopathy and congenital metabolic diseases [40, 41]. Multichannel cEEG monitoring of  
151 neonates at risk of seizures or suspected clinical seizures should be implemented rapidly to  
152 confirm diagnosis and optimise outcomes [42]. Laboratory tests and magnetic resonance  
153 imaging are also required to determine the underlying seizure pathology [43]. A protocol for  
154 laboratory workup in seizures is detailed in a previously published review [25].

155



## 156        **2. Treatment strategies**

157        Once neonatal seizures are suspected, the neonate should be rapidly assessed for treatable  
158        underlying causes, such as hypoglycaemia or electrolyte disturbances [44]. AEDs are then  
159        administered according to clinical preference, independent of seizure cause. AEDs should only  
160        be initiated once seizure activity is confirmed, due to a lack of evidence for any positive  
161        outcomes if they are administered in the absence of seizures [3, 45].

162        As HIE is responsible for the majority of neonatal seizures and seizures are treated with the  
163        same AEDs regardless of underlying injury, the various treatments available for HIE-induced  
164        seizures are reviewed here. Neuro-protective strategies, currently led by therapeutic  
165        hypothermia, are initiated during the latent phase of HIE and may interact with AEDs that are  
166        administered during the secondary phase of HIE, and are therefore briefly mentioned in this  
167        context (Section 3).

### 168                **2.1 Drug treatment for neonatal seizures**

169        Neonatal seizures are neurological emergencies and must be treated promptly since seizures,  
170        particularly high seizure burden, may exacerbate neuronal injury in the immature brain and  
171        contribute to pathogenesis of later cerebral palsy and epilepsy [10, 46, 47]. In neonates with  
172        HIE who do not receive therapeutic hypothermia, there is a peak in seizure burden shortly after  
173        seizure onset (within six hours) [48]. AEDs should ideally be administered within the time  
174        period prior to the peak seizure burden. However, current AEDs are sub-optimal in terms of  
175        effectiveness, safety and long-term outcomes [3, 49] and a systematic review has shown that  
176        the use of AEDs following perinatal asphyxia in the absence of confirmed seizures are of little  
177        benefit with no improvement in survival or neurodevelopmental outcome [45]. AEDs used in  
178        neonates act through a variety of mechanisms to reduce excitability in the brain, thereby  
179        suppressing the seizure. The mode of action of neonatal AEDs is illustrated in Fig. 1.

180 **Insert Figure 1 here**

181

## 2.2 Antiepileptic Drugs: Efficacy, Safety and Tolerability

182  
183 The most frequently used AEDs in both term and preterm babies include phenobarbital,  
184 phenytoin, midazolam, lorazepam, clonazepam, and lidocaine [54]. Current recommendations  
185 suggest initiating anticonvulsant therapies in neonates with phenobarbital, adding either a  
186 benzodiazepine, phenytoin or lidocaine as a second-line agent if seizures continue [3] (Table  
187 1). In a treatment protocol designed by Slaughter *et al.*, a similar treatment regimen is proposed  
188 starting with phenobarbital, followed by levetiracetam, phenytoin or lidocaine, and finally the  
189 addition of a benzodiazepine as a third-line agent [55]. In other studies, if seizures were not  
190 controlled by phenobarbital and/or phenytoin, drugs such as midazolam, clonazepam,  
191 lidocaine, levetiracetam and topiramate have been used [42, 55-57]. A survey of clinicians in  
192 USA found that a majority (73%) would use levetiracetam and/or topiramate despite limited  
193 knowledge about the pharmacokinetics of these drugs in newborn infants [58]. However,  
194 topiramate was shown to exacerbate cell apoptosis caused by phenytoin in rat pups, despite the  
195 absence of neurodegenerative properties when administered as monotherapy [59]. Thus, certain  
196 AED combinations may be detrimental to neurodevelopment. While the use of other AEDs  
197 (carbamazepine, paraldehyde, sodium valproate, vigabatrin, lamotrigine) in the treatment of  
198 neonatal seizures has been described in case reports [60, 61] and recent animal studies have  
199 shown a beneficial anti-seizure effect of potassium channel opener flupirtine in a hypoxia-  
200 model of neonatal seizures [62], we will focus on AEDs that have been recommended in  
201 neonatal treatment protocols and that have been studied in conjunction with EEG monitoring.  
202 AED efficacy is defined differently in many of the studies cited in Sections 2.2, 2.3 and Table  
203 1, but the vast majority state that efficacy is an 80% reduction in seizure severity or complete  
204 seizure cessation, with one notable exception that defined 50% seizure reduction as efficacious  
205 [33]. However, further work is required to define AED efficacy optimally using EEG criteria

206 in view of the well described natural evolution of acute seizures in neonates, particularly those  
207 with HIE [48, 63, 64].

### 208 **2.2.1 Phenobarbital and phenytoin**

209 Phenobarbital remains the first choice of AED in neonatal seizures, due to an extensive history  
210 of its use in this population [3]. Phenobarbital acts by increasing GABA<sub>A</sub> mediated inhibition  
211 [51]. Neonates with persistent seizures are likely to have more severe brain damage and poor  
212 neurodevelopmental outcomes; thus half of the babies on two AEDs and a staggering 95% of  
213 babies on three AEDs were reported to have poor outcomes [47, 65]. Phenytoin, an antiepileptic  
214 that reduces excitatory neurotransmission by blocking a voltage-gated sodium channel, is often  
215 administered second-line to phenobarbital [51]. A Cochrane review found that there was very  
216 little supportive evidence for the main AEDs currently used in the neonatal period, as even  
217 with a combination treatment with phenobarbital and phenytoin, seizures remained in up to  
218 50% of babies, as confirmed by cEEG [42, 49, 66, 67].

### 219 **2.2.2 Lidocaine**

220 Lidocaine acts by inhibiting voltage-gated sodium channels, thereby preventing depolarisation  
221 [50]. Lidocaine is a promising AED in neonatal seizures administered either second-line or  
222 third line with efficacy rates as high as 78%, based on aEEG assessment [68-70]. A very recent  
223 retrospective study of aEEG data has found that lidocaine as a second- or third-line AED had  
224 a good (seizure control for at least four hours) or intermediate (seizure control for at least two  
225 hours) antiepileptic effect in 71.4% of neonates, both term and preterm [70]. An earlier study  
226 demonstrated the lower efficacy rate of 60% with lidocaine, supported by cEEG [42]. One of  
227 the main challenges of using lidocaine in neonates is the risk of adverse events, particularly  
228 with plasma concentrations >9mg/L, including both bradycardia and ventricular tachycardia  
229 [68, 71]. As with many AEDs, a tailored neonatal dosage regimen is needed, as cardio-toxic  
230 levels were found in the majority of neonates treated with a standard lidocaine infusion [68].

231 A neonate-specific regimen was designed using pharmacokinetic modelling, and optimal  
232 lidocaine plasma levels were achieved in the majority of treated full-term neonates [68].  
233 Furthermore, lidocaine dosing was studied in both term and preterm neonates, and it was found  
234 that both cohorts of neonates should receive approximately 50% of the previously  
235 recommended dose i.e. a 1 kg neonate should receive 52mg as opposed to 110mg [72].  
236 However, lidocaine demonstrated a good safety profile in neonates [73].

### 237 **2.2.3 Benzodiazepines**

238 Benzodiazepines have had varied success as second- and third-line agents in the treatment of  
239 neonatal seizures. Benzodiazepines allosterically modulate the chloride channel in the GABA<sub>A</sub>  
240 receptor to increase inhibitory neurotransmission [51]. Midazolam response rates vary from 0-  
241 100%, with both 0% and 100% efficacy being observed using cEEG monitoring (see Table 1)  
242 [42, 67]. Efficacy rates measured by aEEG are reported as 50% when midazolam is used as a  
243 second-line AED, increasing to 73-100% when administered as a third-line AED [69, 74].  
244 Midazolam appears to be less effective than lidocaine at treating persistent seizures,  
245 particularly those caused by the most severe form of HIE [69, 75].

246 The evidence for the effect of other benzodiazepines used in neonatal seizures is less  
247 convincing [55]. Clonazepam did not abolish any seizures as a second-line AED in three  
248 neonates monitored by cEEG [42]. The support for lorazepam as an AED is sparse, with less  
249 than half of the studied neonates monitored by cEEG [76, 77]. Seizure control rates were as  
250 high as 86% and 100% in two studies, but these results are unreliable due to the absence of  
251 cEEG monitoring [76, 77].

### 252 **2.2.4 Levetiracetam**

253 Levetiracetam is a relatively new AED which is proposed to act through synaptic vesicle  
254 glycoprotein 2A (SV2A) which is a protein thought to be involved in the release of

255 neurotransmitters [78]. Levetiracetam is efficacious in treating various seizures in both adults  
256 and children. In addition, levetiracetam has a very favourable pharmacokinetic and safety  
257 profile in neonates [79, 80]. Levetiracetam has demonstrated some efficacy as a neonatal and  
258 paediatric AED, according to cEEG findings which show 35-64% efficacy within 24 hours,  
259 rising to improvements in 52-100% of patients in 72 hours [33, 56]. Levetiracetam was initiated  
260 as a second- or third- line AED in the majority of recorded cases [33]. Evidence from  
261 randomised-controlled trials is needed to endorse levetiracetam as a safe and effective AED. A  
262 trial is ongoing in America looking at the safety, efficacy and pharmacokinetic profile of  
263 levetiracetam in neonates (NCT01720667), with more efficacy/safety trials planned in France  
264 (NCT02229123) and China (NCT02550028) [38].

### 265 **2.2.5 Topiramate**

266 Topiramate reduces the frequency of action potential firing by altering GABA  
267 neurotransmission, blocking voltage-gated sodium channels and by weakly blocking AMPA  
268 glutamate receptors [81]. Similar to levetiracetam, pharmacokinetic and safety profiles are  
269 favourable, but little is known about the safety, efficacy or pharmacokinetics in a critically-ill  
270 newborn population [57]. In a small, retrospective study, topiramate was considered an  
271 effective add-on agent in neonatal seizures in four out of six neonates, and no major safety  
272 concerns were highlighted [57]. However, this study was limited by the lack of EEG  
273 monitoring [57].

## 274 **2.3 Potential adjunct antiepileptics**

### 275 **2.3.1 Bumetanide**

276 Bumetanide is a potential adjunct to AED treatments for neonatal seizures [82]. A number of  
277 years ago, bumetanide was observed to have antiepileptic effects in kainic acid-induced  
278 seizures *in vivo* [83]. This was believed to be due to its ability to block ion cotransporters in  
279 neurons and glia of the central nervous system, which in turn affected GABA signalling [83].

280 Bumetanide blocks NKCC co-transporters, NKCC1 and NKCC2, which both move chloride  
281 into cells [84]. Bumetanide was originally developed as a loop diuretic, which reduces oedema  
282 by inhibiting the reabsorption of sodium, potassium and chloride through NKCC2 in the thick  
283 ascending loop of Henle of the kidney [84]. Bumetanide also inhibits NKCC1, an isoform of  
284 the NKCC cotransporter that is widely expressed, including on neurons in the brain [84].  
285 GABA is excitatory in immature neurons due to the accumulation of chloride through NKCC1  
286 [85]. By preventing intracellular chloride build-up, bumetanide is thought to decrease or even  
287 reverse the excitatory action of GABA, thus presenting a potentially useful combination  
288 therapy with GABAergic anticonvulsants [64, 82]. There are gaps in our knowledge of this  
289 potential adjunct to AEDs for the treatment of neonatal seizures, namely the dose at which it  
290 acts in the brain, the human blood-brain barrier permeability/transport of bumetanide as well  
291 as its effect on development of the central nervous system (CNS). Two clinical trials were  
292 initiated to establish the safety and efficacy of bumetanide in neonatal seizures, one in Europe  
293 (NCT01434225) and one in the USA (NCT00830531) [38]. In the European dose-finding  
294 clinical study, bumetanide was administered according to a bivariate Bayesian sequential dose-  
295 escalation design, in which participants were treated with four doses of bumetanide  
296 (0.05mg/kg-0.3mg/kg) each given twelve hours apart, with the first dose given in conjunction  
297 with phenobarbital [64]. However, the trial was concluded early as the benefit: risk ratio was  
298 no longer favourable and the efficacy endpoint was not achieved in any of the trial participants  
299 [64, 86]. It has been suggested that this is partially due to a poor CNS effect of bumetanide at  
300 the doses used and evidence to corroborate this have come from animal studies that indicate a  
301 poor brain permeability of bumetanide [87]. Many studies are examining novel ways to  
302 enhance brain levels of bumetanide in an effort to overcome the pharmacokinetic issues  
303 hindering its therapeutic success [87-90].

**Table 1: Drug treatments of neonatal seizures-efficacy, safety and tolerability**

Drug	Place in treatment protocol	Efficacy	Safety	Tolerability
Phenobarbital	First-line.	Effective in 43% of neonates in a randomised controlled trial (n = 30) [66]. Phenobarbital achieved seizure control in 50% of neonates (n = 22) [42], and 47% of neonates in a further study (n = 32) [67]. Cost-effective AED [91].	May impair neurodevelopment and increase apoptosis of neurons [92]. Potential for drug-drug interactions.	Many CNS side-effects i.e. sedation, impaired cognition, depressed mood [91].
Phenytoin	Second-line.	Response in 45% of neonates to a dose that achieves a free plasma concentration of 3µg/mL (n = 29) [66].	Concerns about potential detrimental effect on developing neurons [60]. Potential for drug-drug interactions [55].	No changes in cardiac or respiratory function observed [66]
Levetiracetam	Emerging. Second- or third-line [3, 55]. Effect may be additive with other AEDs [93].	Effective with twice daily dosing. Efficacious in 82% of preterm neonates with seizures (n=11) [94]. Achieves full control of seizures in 33% (n = 18) [95], 35% (n = 23) [33] and between 32% and 100% of cases, depending on the treatment duration (n = 22) [56].	Does not cause neuronal apoptosis in rat pups [93].	Side effects in infants and children: somnolence and irritability.  Well tolerated [79].
Lidocaine	Second- or third-line.	Response rate varies from 60% (n = 5) [42], to 71.4% (n = 413) [70], to 76% (n = 20) [68] and 77% (n = 22) [69]. Optimised dosing regimen achieved seizure control in 78% neonates (n = 15) [68].	Cardiac toxicity i.e. bradycardia-increased risk following other cardio-toxic agents e.g. phenytoin.	Risk of arrhythmias in 5% patients [68].
Midazolam	Second-line.	Reported response to treatment shows a wide variability from no response (n = 3) [42] to 50% (n = 8) [69] to 73% (n = 15) [74] to 100% response (n = 13) [67]. Improved neurodevelopment at 1 year of age compared to non-responders [67].	Higher doses or combination treatment with hypothermia may cause cardiac depression [69, 75]. Short-term drowsiness observed [67].	Well tolerated, no serious adverse effects noted [67].
Topiramate	Emerging.	Efficacy studies in neonates ongoing [81]. Efficacy of 67% in one small, retrospective study (n = 6), but no EEG monitoring so unreliable [57]. Hypothesised to have synergistic neuro-protective effects in neonates; reduces brain injury in animal models of HIE [96].	Seems safe- no increase in risk of death, short-term detrimental effects or gross brain pathology [97].	Well-tolerated, no adverse effects noted [96]



Bumetanide	Emerging.	Low efficacy rates of ~36% (n = 14) with research protocol doses of between 0.05 mg/kg and 0.3 mg/kg given 12 hours apart for a total of four doses-rescue AEDs were required by most neonates and efficacy endpoint not met by any trial participants [64, 86].	Potential risk of ototoxicity, especially if given concomitantly with other ototoxic drugs [64]. Other side effects include dehydration.	Well tolerated up to 0.1mg/kg dose [64].
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305

306

#### 2.4 Pharmacokinetic properties of AEDs

307 There are a variety of physiological differences between neonates and adults. These variations  
308 in physiology affect all pharmacokinetic processes in the neonate, including absorption,  
309 distribution, metabolism and elimination [60]. These variations are detailed in a review by  
310 Alcorn *et al.*, but the salient changes are noted in Table 2. Key pharmacokinetic parameters,  
311 including volume of distribution ( $V_d$ ), fraction unbound in plasma ( $f_u$ ), clearance (Cl) and  
312 elimination half-life ( $t_{1/2}$ ), are different in neonates compared to adults. Moreover, there is wide  
313 variability in these pharmacokinetic parameters within the neonatal population, as can be seen  
314 by the ranges reported (Table 3).

315

316 **Table 2: Physiological differences between neonates and adults**

Stage	Pharmacokinetic parameter	Neonate	Adult
Absorption	Gastric pH	6-8	2
	Gastric emptying time	Reduced rate	-
	Intestinal Motility	Reduced rate	-
Distribution	Body composition		
	<ul style="list-style-type: none"> <li>❖ Water</li> <li>❖ Fat</li> </ul>	74% 14%	55-60% ~20%
	Plasma proteins		
	<ul style="list-style-type: none"> <li>❖ Albumin</li> <li>❖ <math>\alpha</math>1 acid glycoprotein</li> </ul>	~75% ~25%	100% 100%
Metabolism	Enzyme expression		
	<ul style="list-style-type: none"> <li>❖ Foetal                             <ul style="list-style-type: none"> <li>○ ST, GST</li> </ul> </li> <li>❖ Early Neonatal                             <ul style="list-style-type: none"> <li>○ UGT, NAT</li> </ul> </li> </ul>	0-10% 25-37.5%	100% 100%
	Cytochrome P activity		
	<ul style="list-style-type: none"> <li>❖ Foetal                             <ul style="list-style-type: none"> <li>○ 3A7</li> </ul> </li> <li>❖ Early neonatal                             <ul style="list-style-type: none"> <li>○ 2D6</li> <li>○ 2E1</li> </ul> </li> <li>❖ Neonatal                             <ul style="list-style-type: none"> <li>○ 1A2</li> <li>○ 2C</li> <li>○ 3A4</li> </ul> </li> </ul>	500-600% 4-24% 21-36% 5-20% 2-42% 3-29%	100% 100% 100% 100% 100%
	Bacterial flora	Very limited	100%
Excretion	Glomerular filtration rate	~90%	100%
	Tubular secretion	~50%	100%
	Renal bloodflow	~65%	100%

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318

319

320 **Table 3: Drug treatments of neonatal seizures from published studies-pharmacokinetics**

AED	Dose	t <sub>1/2</sub> (h)	f <sub>u</sub> (%)	Cl (L/hr/kg)	V <sub>d</sub> (L/kg)	Ref
Phenobarbital	D <sub>L</sub> : 20mg/kg (twice if required) D <sub>M</sub> : 5mg/kg/day	73.9-154.5	57-64	0.0053-0.0141	0.64-1.17	[55, 66, 99-102]
Phenytoin	D <sub>L</sub> : 20mg/kg D <sub>M</sub> : 5mg/kg/day	Wk 1: 20.7 ± 11.6 Wks 2-4: 7.6 ± 3.5	19.8 ± 2.6	0.00151-0.139	0.8 ± 0.26	[55, 103, 104]
Levetiracetam	D <sub>L</sub> : 10-50 mg/kg D <sub>M</sub> : 10-80 mg/kg/day (mean 45 mg/kg/day)	Day 1: 18.5 ± 7.1 Day 7: 9 ± 2	~100	0.042-0.078	0.89-1.01	[33, 55, 56, 79, 95]
Lidocaine	D <sub>L</sub> : 2mg/kg D <sub>M</sub> (normothermia): 5-7 mg/kg/h for 4 h, 2.5-3.5 mg/kg/h for 6-12 h, 1.25-1.75 mg/kg/h for 12 h *Altered D <sub>M</sub> recommended in hypothermia-see ref. 54	5.2-5.4	20-40	0.462-1.68	3-3.2	[54, 60, 68, 72]
Midazolam	D <sub>L</sub> : 0.05-0.15mg/kg D <sub>M</sub> : 0.06-0.4 mg/kg/h	6.9	3.1	0.124	1-1.7	[55, 105]
Topiramate	D <sub>M</sub> : 5mg/kg-10mg/kg daily	35.6 ± 19.3	~85	0.0156 ± 0.0048	0.6-1	[60, 81, 96, 102]

321 <sup>a</sup> t<sub>1/2</sub>: half-life; f<sub>u</sub>: fraction unbound; Cl: clearance; V<sub>d</sub>: volume of distribution; D<sub>L</sub>: Loading dose; D<sub>M</sub>: Maintenance dose; Wk: week.

322

### 323 **3. Combining therapeutic strategies**

#### 324 **3.1 Adjunct therapies in HIE with potential for interaction with antiepileptics**

##### 325 **3.1.1 Hypothermia**

326 Hypothermia has demonstrated neuro-protective properties in neonates with moderate to severe  
327 HIE [107-109]. Since the introduction of therapeutic hypothermia, the composite risk of death  
328 and major disability has been reduced by approximately 25% [108]. Neurological outcomes in  
329 cooled neonates with HIE improved at both 18 months and six-seven years of age [108, 109].  
330 Hypothermia significantly reduces seizure burden, as measured by cEEG, in neonates with HIE  
331 [110, 111]. Seizure burden during hypothermia is characterised by a more even distribution  
332 over time (as opposed to the accumulation seen at seizure-onset in normothermia) and de-novo  
333 seizures may occur after re-warming [10, 48, 63]. It has been proposed that hypothermia should  
334 also be tested as a therapeutic strategy in late premature neonates with HIE and neonatal stroke,  
335 both of which can also result in seizures [112].

##### 336 **3.1.2 Emerging neuro-protective treatments**

337 Additional neuro-protective strategies that are emerging include xenon, erythropoietin,  
338 melatonin, allopurinol and sevoflurane [113-119]. Thus far, the authors have found no reports  
339 of combination treatment with AEDs and emerging neuro-protective drugs. However, the  
340 combination of these novel neuro-protective agents, hypothermia and AEDs are a definite  
341 possibility in the future. Briefly, xenon protects the brain from excitatory injury by  
342 antagonising the N-methyl-D-aspartate (NMDA) glutamate receptor reducing total  
343 neurotransmission and is currently under investigation in a Phase 2 trial [120]. Erythropoietin  
344 has anti-inflammatory properties and is also anti-apoptotic [121, 122]. It has been shown to  
345 reduce detrimental neurodevelopmental outcomes in neonates with moderate-severe HIE  
346 [123]. Melatonin reduces oxidative stress through a variety of mechanisms, such as scavenging

347 oxygen free radicals and has been shown to augment neuroprotection by hypothermia in a  
348 piglet model of HIE [117]. Sevoflurane reduced hippocampal apoptosis in a rat model of  
349 intrauterine perinatal asphyxia and thus may be neuroprotective [113]. Allopurinol was found  
350 to have anti-oxidant properties [116].

### 351 **3.1.3 Sedation**

352 Intravenous morphine is commonly used as a sedative during hypothermia, as it reduces pain  
353 and stress, allows the patient to tolerate hypothermia and can be titrated to optimal response  
354 [124]. In a preclinical model of HIE, hypothermia without sedation lacked neuro-protective  
355 properties [125]. In a small group of term and preterm neonates without underlying brain  
356 injury, morphine infusion at a rate of 10-20 mcg/kg/hour was found to be associated with  
357 excessive epileptiform activity on cEEG [126].

358 It is known that morphine clearance is decreased during hypothermia, resulting in an increased  
359 concentration of morphine in both cerebrospinal fluid and plasma [127, 128]. In terms of  
360 pharmacodynamic considerations, the affinity of morphine for its receptor appears reduced in  
361 hypothermia, but the incidence of hypotension is increased [127, 129]. Neonates with HIE who  
362 are sedated with opioids show less brain injury and display better outcomes [130]. Little is  
363 known about drug-drug interactions with AEDs, but it is advised that barbiturates such as  
364 phenobarbital may increase the sedating effect of opioids [131].

### 365 **3.2 Antiepileptics and hypothermia**

366 It is thought that synergistic therapy including a traditional AED and hypothermia may  
367 augment neuro-protective properties of either treatment given alone [132]. Combination  
368 treatments need to be explored further to complement this claim. However, caution needs to be  
369 exercised as hypothermia may alter pharmacokinetics of AEDs in neonates by decreasing  
370 absorption, distribution or metabolism/clearance [100, 133-135]. Moreover, as multi-organ

371 dysfunction is frequently a characteristic of HIE, the combination of therapeutic hypothermia  
372 and organ impairment, particularly renal and hepatic, may have additive detrimental effects on  
373 fundamental pharmacokinetic processes [136]. The rewarming phase following hypothermia is  
374 another period of pharmacokinetic and pharmacodynamic uncertainty and is likely to be a  
375 window of time in which serious toxicity and adverse reactions could occur, due to a lag time  
376 between the return of normal metabolic enzyme and transporter function [135]. There have  
377 been reports of seizures re-occurring during the rewarming phase, but the affected infants were  
378 not receiving regular AEDs [10, 137]. Thus, combination treatment with hypothermia and  
379 AEDs may be useful, but must be approached with caution due to uncertainties regarding the  
380 effect of hypothermia on efficacy, safety and pharmacokinetics of such medications. It is  
381 important to identify AEDs, doses and dosage intervals that are suitable for neonates during  
382 and after hypothermia.

### 383 **3.2.1 Phenobarbital and hypothermia**

384 Positive synergism of first-line AED phenobarbital and hypothermia was observed in a rodent  
385 model of HIE, with both early and late assessment of neuropathology and sensorimotor  
386 performance demonstrating improvements [138]. However, current evidence suggests that this  
387 combination has not translated to a reduced risk of death or brain damage in neonates [139,  
388 140]. Seizures were detected using aEEG, and a 66% reduction in seizures was demonstrated  
389 for neonates treated with hypothermia and with plasma concentrations of phenobarbital above  
390 20mg/L [140].

391 The pharmacokinetics of phenobarbital were examined in hypothermic critically-ill neonates  
392 [100, 101]. It was found that minimum, maximum and average plasma concentrations were all  
393 larger in cooled neonates versus normothermia [100]. However,  $V_d$  and clearance remained  
394 unchanged [101]. It was concluded the alterations in pharmacokinetics of phenobarbital during  
395 hypothermia in neonates were not clinically significant, and that a total maximum dose of

396 40mg/kg can be safely administered in hypothermia prior to initiation of second-line AED  
397 [140]. In contrast, metabolism of phenobarbital via CYP2C19 was significantly reduced when  
398 it was administered to critically injured children who were cooled under more severe  
399 hypothermic conditions to 30-31°C [135, 141]. Therapeutic drug monitoring of phenobarbital  
400 allows for tight control of AED concentrations, which may be particularly important during  
401 hypothermia.

### 402 **3.2.2 Lidocaine and hypothermia**

403 Lidocaine was administered as a third-line anticonvulsant to neonates undergoing hypothermia  
404 treatment for asphyxia-induced seizures with aEEG monitoring. An impressive 91% of these  
405 patients responded to lidocaine [134]. This is a similar response rate to that observed in  
406 normothermic babies [42].

407 The pharmacokinetics of lidocaine are altered by hypothermia. Clearance of lidocaine is  
408 reduced by 24% as hepatic blood flow is reduced during hypothermia [134]. Despite these  
409 changes, no cardiotoxicity was observed in hypothermic neonates when an altered dosing  
410 regimen, equating to 70% of the total lidocaine dose given to normothermic neonates, was  
411 administered [134].

### 412 **3.2.3 Topiramate and hypothermia**

413 Animal studies suggested that the combination of topiramate and hypothermia improved motor  
414 and brain tissue damage in a model of HIE, where neither drug alone conferred any  
415 neuroprotection [142]. In neonates, there were no statistically significant changes in survival  
416 rate or brain damage observed when topiramate was given in combination with hypothermia  
417 when compared to hypothermia alone [97]. A randomised-controlled trial of topiramate and  
418 hypothermia in combination is underway, which will examine efficacy of seizure control with  
419 this treatment strategy (NCT01765218) [38, 81].

420 The pharmacokinetic profile of topiramate is altered when administered during hypothermia  
421 treatment: maximum, minimum and average concentrations,  $t_{1/2}$  and area under the  
422 concentration-time curve are significantly higher in hypothermia [96]. However, these  
423 pharmacokinetic variations are not clinically significant, and the majority of neonates are  
424 observed to have topiramate concentrations within the safe, effective concentration range [96].

#### 425 **3.2.4 Midazolam and hypothermia**

426 The efficacy of midazolam as a second-line AED in seizing neonates undergoing hypothermia  
427 treatment is modest, achieving seizure control in only 23% of neonates, confirmed using aEEG  
428 monitoring [75].

429 The pharmacokinetic profile of midazolam in neonates were not significantly changed by  
430 hypothermia [75, 143]. However, the incidence of midazolam-induced hypotension increased  
431 in neonates undergoing therapeutic hypothermia [75]. Midazolam levels in the serum of  
432 asphyxiated infants (both normothermic and hypothermic) were found to be highly variable  
433 and unpredictable, due to renal/hepatic impairment caused by the initial injury [143].  
434 Furthermore, it is worth noting that the combination of midazolam and hypothermia in adults  
435 with disorders of the CNS gave rise to significant decreases in clearance and increases in  $V_d$  of  
436 midazolam compared to midazolam treatment alone [144].

#### 437 **3.2.5 Bumetanide and hypothermia**

438 Bumetanide pharmacokinetics including clearance and  $V_d$  were calculated in a neonatal  
439 population [64]. These patients were also receiving hypothermia treatment and phenobarbital.  
440 Clearance values appear to generally be in agreement with values previously reported in a  
441 neonatal population [145, 146]. The combination of phenobarbital, bumetanide and  
442 hypothermia in a neonatal population with HIE-induced seizures was not effective, as none of



443 the neonates achieved the requisite 80% seizure reduction without the need for rescue AEDs  
444 [64, 86].

### 445 **3.2.6 Phenytoin and hypothermia**

446 There are no data from neonates with seizures on the efficacy or safety of phenytoin and  
447 hypothermia together. However, there are reports from trials on the use of phenytoin and  
448 hypothermia in older children aged 2-16 years, as well as adult patients, for the treatment of  
449 traumatic brain injury [147, 148]. In these populations, decreases in metabolism by CYP2C9  
450 and CYP2C19 resulted in reduced clearance compared to values obtained after rewarming  
451 [135, 147, 148]. In children, it was also found that increased phenytoin levels are present both  
452 during and after rewarming which increased the risk of drug toxicity even after hypothermia  
453 had finished [147]. Moreover, a case report has described an additive bradycardic effect of  
454 therapeutic hypothermia and phenytoin [149]. Hypothermia in this case occurred during  
455 surgery and was not controlled. The authors hypothesise that the cardiac depressant effects of  
456 both treatments acted synergistically and that extreme caution should be exercised when co-  
457 administration is necessary [149].

458

#### 4. Knowledge gaps

459  
460 There is an urgent need for more randomised controlled trials in neonates to validate a treatment  
461 algorithm for seizures, especially when used in combination with hypothermia. Due to a lack  
462 of evidence from clinical studies, seizure treatments consist of older generation drugs that have  
463 more side-effects than newer drugs [3]. In general, efficacy rates of treatments are  
464 underwhelming (Table 1). Furthermore, there is a need to observe long-term  
465 neurodevelopmental outcomes following each of the proposed treatments, and to define the  
466 optimal length of time to continue with AED therapy given the concern regarding their effect  
467 on long-term brain development [16, 150].

468 There is a paucity of data on the pharmacokinetics and efficacy of many AEDs used in  
469 neonates, including levetiracetam, lidocaine and topiramate [151]. There are also major gaps  
470 in our knowledge about the efficacy and safety of most anticonvulsant drugs, particularly in  
471 preterm neonates.

472 In HIE it is imperative that the pharmacokinetics of AEDs during both active therapeutic  
473 hypothermia and the rewarming phase in neonates with seizures are elucidated, particularly  
474 with regards to phenytoin, a popular second-line AED [135]. Furthermore, drug-drug  
475 interactions are significantly under-investigated, especially co-administration of AEDs with  
476 novel neuro-protective drugs such as xenon, allopurinol, melatonin and erythropoietin.

477 Multichannel EEG is essential to accurately measure the efficacy of AEDs [24]. The optimal  
478 algorithm for detecting seizures remains to be developed, to enhance bedside recognition of  
479 seizures by non-EEG experts.

480 In the last decade there has been an increased interest in developing safe and effective drugs  
481 for neonates. The European Commission through the FP7 Framework promoted research on  
482 the safe and effective use of medicine in children by specifically supporting applications for

483 the neonatal age group [152]. More recently, the International Neonatal Consortium was  
484 launched in May 2015. This is a consortium of stakeholders focused on the development of  
485 effective medicines for neonates, including the Food and Drug Administration (FDA),  
486 European Medicines Agency (EMA), the pharmaceutical industry, academia, patient research  
487 groups, and family advocates. The consortium aims to align priorities and initiate  
488 collaborations to accelerate the development of safe and effective treatments for neonates. One  
489 of the first topics that this consortium has prioritised for further development is the treatment  
490 of neonatal seizures.

## 491 **5. Conclusion**

492 The treatment of neonatal seizures remains sub-optimal. Treatment algorithms are based on  
493 minimal trial data on older generation drugs. Phenobarbital remains the first-line antiepileptic  
494 of choice, despite suboptimal efficacy and altered underlying pharmacodynamics in the  
495 immature brain. However, there is no consensus on a replacement first-line drug or even on the  
496 most efficacious and suitable second-line AED. A lack of randomised controlled trials to guide  
497 treatment regimens in neonatal seizures is halting progress in the field. There are a multitude  
498 of drug-drug and drug-hypothermia interactions that remain to be elucidated, including the  
499 efficacy/safety of antiepileptic polypharmacy in neonates. These knowledge gaps have been  
500 identified and urgently need to be bridged by designing and conducting high quality clinical  
501 trials in neonates. Until the pharmacokinetic/pharmacodynamic profiles of antiepileptic  
502 medications in hypothermia are sufficiently investigated, therapeutic drug monitoring of serum  
503 antiepileptic levels is encouraged. The efficacy of antiepileptic treatment protocols should  
504 always be measured using cEEG monitoring.

505

506

507

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511

512 *Conflict of Interest*

513 Maria Donovan, Brendan Griffin, Liudmila Kharoshankaya, John Cryan and Geraldine Boylan  
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515

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943 **Fig. 1 Mode of action of neonatal AEDs**

944 Many drugs act by reducing excitatory neurotransmission (glutamatergic synapse). Phenytoin,  
945 lidocaine and topiramate prevent depolarisation by inhibiting voltage-gated sodium channels  
946 [50, 51]. Levetiracetam prevents calcium influx through N-type calcium channels which in turn  
947 reduces exocytosis and reduces the release of glutamate from intracellular vesicles by  
948 modulating synaptic vesicle protein 2A (SV2A) [51, 52]. On the postsynaptic terminal,  
949 phenobarbital and topiramate reduce excitatory neurotransmission via the AMPA/kainate  
950 glutamate receptor [51, 53]. Anticonvulsants including phenobarbital, benzodiazepines and  
951 topiramate work by enhancing inhibitory neurotransmission via the GABA<sub>A</sub> receptor  
952 (GABAergic synapse) [51]. Bumetanide can alter the action of GABAergic agents by  
953 preventing intracellular accumulation of chloride through NKCC1 [17].

954 (AMPA =  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA = N-methyl-D-  
955 aspartate; GABA =  $\gamma$ -amino butyric acid; GAD = glutamic acid decarboxylase; SV2A =  
956 synaptic vesicle protein 2A)

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