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ORIGINAL ARTICLE

Altered sleep and inflammation are related to outcomes in neonatal encephalopathy

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Abstract

Aim: Immune dysregulation and delayed onset of sleep wake cycling (SWC) are associated with worse outcome in neonatal encephalopathy (NE), however the association between sleep and immune dysfunction in NE remains unclear. Aimed to evaluate association of sleep and systemic inflammation with outcomes in NE.

Methods: Amplitude-integrated electroencephalography (aEEG) recordings were collected on infants undergoing therapeutic hypothermia (TH). Duration to onset of (SWC) and sleep quality (SQ) were examined. Blood samples collected during the first 2 days of life. Thirteen pro- and anti-inflammatory serum cytokines were quantified. Adverse outcome defined as death or abnormal MRI brain.

Results: Earlier onset of SWC and better SQ had less adverse outcomes. SQ provided better prognostic value and showed better interobserver agreement compared

Abbreviations: AEEG, amplitude integrated electroencephalography; BG, basal ganglia; CHI, Childrens Hospital Ireland; ELISA, enzyme linked immunosorbent assay; HPA, hypothalamic-pituitary-axis; IL, interleukin; IQR, interquartile range; NE, neonatal encephalopathy; NICU, neonatal intensive care unit; PBS, phosphate buffered solution; SQ, sleep quality; SWC, sleep wake cycling; T, thalamus; TH, therapeutic hypothermia; WS, watershed.

to duration to SWC. Better SQ associated with lower cytokines EPO and interleukin (IL)-1β. In infants with unfavourable outcome, shorter duration to SWC was associated with higher EPO and better SQ was associated with lower TNF-α.

Conclusion: Earlier onset of SWC or better SQ showed less systemic inflammation and fewer adverse outcomes. SQ during TH provided better prognostic information than time of onset of SWC. Modulation of circadian rhythm in infants with NE may have an immunomodulatory role, leading to improved outcomes.

KEYWORDS
brain injury, cytokines, inflammation, neonatal encephalopathy, neuroimaging, sleep

1 | INTRODUCTION

There is a potent reciprocal connection between sleep and the immune system.[1] Both sleep and the immune system are disrupted in neonatal encephalopathy (NE).[2,3] Many factors that promote sleep and entrain the circadian rhythm are disrupted during care the neonatal intensive care unit (NICU)[4] Both sleep disruption and dysregulated inflammation are targets for intervention for newborn infants with NE.

Dysregulated inflammation is key process in brain injury in NE.[3] Several studies have demonstrated altered inflammatory cytokine production in NE,[3–6] and dynamic changes in inflammatory cytokines correlated with improved outcomes in trials of therapeutic hypothermia.[7] Therefore several immune-modulating agents are under investigation as adjunctive therapies for neuroprotection in NE.[3,8]

Sleep promotes host defence and regulates the production of inflammatory cytokines, many of which also act as potent somnogens to enhance sleep.[9,10] Sleep exerts an effect on effector systems such as the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system to regulate the release of cortisol, a potent anti-inflammatory mediator and to modulate the release of noradrenaline, a key modulator of the innate immune system that promotes activation of the inflammatory response. Sleep disruption is associated with dysregulated inflammation including increased production of pro-inflammatory cytokines,[11] and adverse outcomes.[1,11] Sleep also promotes brain development by providing support for changes in brain activity, promoting glymphatic function in clearing toxins from the brain[12] and reducing DNA damage and potentially promoting DNA repair.[13] There is evidence of sleep disruption in infants with NE, with longer duration to sleep wake cycling (SWC) being associated with adverse outcome in NE.[2] While it is not fully understood what promotes sleep in the neonatal period, the most likely factors are light exposure primarily, feeding and, less significantly, social cues.[14,15] These are all disturbed in infants undergoing TH in NICU.[14,16,17]

Circadian clocks are also a major regulator of all aspects of immune function and circadian disruption has been associated with dysregulated inflammatory responses.[18,19] Foetal circadian rhythms in gene expression and the production of hormones including melatonin, dopamine and glucocorticoids are entrained to and coupled with maternal signals by 28–30 weeks of gestation.[20] Newborns display SWC as early as the first 6 h of life,[21] however it takes at least 10–12 weeks for sleep cycles to display diurnal rhythms.[22] Circadian rhythms are gradually established in the early infantile period.[23]

Key notes

- Both sleep quality (SQ) and duration to onset of sleep wake cycle (SWC) provide excellent prognostic information in infants with neonatal encephalopathy (NE) undergoing therapeutic hypothermia (TH).
- SQ provide better prognostic information than duration to onset of SWC in infants with NE undergoing TH.
- Sleep disruption is associated with increased immune dysregulation in infants with NE.

Although both sleep and inflammation responses are disrupted in NE, it remains unclear whether there is a causal relationship. Regardless, both are potentially modifiable if a causal relationship is established. Many factors that promote sleep and entrain the circadian rhythm are disrupted in the NICU.[14] In preterm infants cycled light has demonstrated improved outcomes compared to continuous dim lighting.[24] Many immunomodulatory agents are currently being investigated as adjunctive therapies in NE[25] although none are currently used in routine practice. Improved understanding of sleep and circadian control of the immune system in NE may be useful in modulating dysregulated inflammation associated with brain injury.

2 | METHODS

2.1 | Study population

Infants with moderate–severe NE being treated with therapeutic hypothermia (TH) were prospectively recruited between August 2016 and January 2019 as part of the NIMBUS study.[5,26] Recruitment took place across Dublin's three level three neonatal intensive care units (NICUs) which are national centres for TH and which combined have approximately 27 000 deliveries per annum. Infants were

eligible for inclusion if they were diagnosed with NE and met the criteria previously published.[5] Infants with NE were classified by severity of neurological dysfunction according to Sarnat staging.[27] The anticonvulsant treatment used was as follows: Phenobarbitone ($n=20$), leviteracitem $n=(10)$, midazolam ($n=8$), phenytoin ($n=6$), clonazepam ($n=0$). There were no babies with positive blood cultures and no late onset sepsis, although one baby had meningitis. There were 14 abnormal placental histology of which 4 were chorioamnionitis. Infants born prematurely at <35 weeks gestation, infants with major congenital abnormalities or infants born to mothers with a history of substance misuse were excluded. Fully informed written consent was obtained from the parents of each participant following institutional ethical approval and engagement with the Parents participation forum of the overall project. This study was part of a larger project the Neonatal Inflammation and Multiorgan dysfunction and Brain injury research project and was funded at the Health research Board Neonatal Encephalopathy multidisciplinary PhD training network (NEPTUNE: www.nbci.ie).[26,28]

2.2 | Sampling and cytokine analysis

Blood samples were taken from enrolled infants and collected in sodium citrate bottles during routine clinical phlebotomy during the first 4 days of life and brought to the laboratory for immediate processing. Samples were taken at the following time points: 1–24 h and at 72–96 h, following a strict standardised SOP. When available, samples were collected from umbilical or peripheral arterial lines, or otherwise by peripheral venous sampling. Samples were treated with phosphate-buffered solution (PBS) and incubated for 1 h at 37°C. Serum was isolated from the whole blood samples by centrifugation and stored in the freezer at −80°C until batch analysis by enzyme linked immunosorbent assay (ELISA). Customised plates for this study and selected cytokines were prepared by MesoScale Discovery (www.meso-scale.com). Plates were analysed on the Sector Imager and validated. All cytokines are measured in pg/mL. Measured cytokines included EPO, GM-CSF, IFNγ, IL-1α, IL-1RA, IL-1β, IL-6, IL-8, IL-10, IL-18, TNF-α, TNF-β and VEGF.

Cytokines are part of a paper under submission. The results are part of a paper on an in vitro examination of melatonin and therefore were not included in this paper. Cortisol was not measured in this sample as immunosassay is not considered sufficiently accurate. Improved specificity and sensitivity can be achieved by mass spectrometry coupled with chromatographic separation methods, which is a cutting-edge technology to measure individual as well as a panel of steroids in a single analytical run.[29] The use of erythropoietin as an adjunct therapy did not occur in this study.

2.3 | aEEG recording and analysis

Amplitude-integrated electroencephalography (aEEG) recordings were routinely started within a few hours for all infants treated with TH for NE. Data was collected from electrodes placed over the frontotemporal and parietal regions for assessment. These recordings were started prior to TH and lasted for the duration of TH. They were subsequently stored on CD discs for later review. aEEG recordings for all infants were retrospectively reviewed by two reviewers independently (TH and PS) who were blinded to infant outcome. Where differences in assessment arose following independent review of aEEG recordings by each reviewer, consensus was agreed following a third combined aEEG review. Time from birth to onset of aEEG recording was documented when sufficient infant data was available. aEEG recordings were reviewed from the earliest available time until the presence of SWC was detected, defined by Takenouchi et al. as the 'presence of features of both wakefulness or active sleep and quiet sleep with at least two clear state changes during a 6-h epoch'.[30] The aEEG recordings were then reviewed a second time and SQ was scored according to the classification by Burdjalov et al.[31] This scoring system graded electrographic evidence of sleep according to the presence of continuity, the presence of cycling, amplitude of the lower border and bandwidth span and amplitude of the lower border. A stable 4-h epoch was selected to be analysed using component variables of the aEEG scoring system. All variables were scored according to the individual component scores. This revealed a total sum for each recording of each individual infant. The minimal possible scoring was 0 and the maximum score was 13.

Our neonatal unit did not have a circadian day and night protocol at this time but there was a quiet 2 h period from 3 to 5 PM with low lighting, and the evening shift for 12 h had low level lighting.

2.4 | MRI acquisition and analysis

Adverse outcome was defined as death or abnormal MRI brain. Surviving infants underwent MRI within the first 2weeks of life in one of two national paediatric referral centres in Ireland, Children's Hospital Ireland (CHI) at Crumlin or CHI at Temple Street or when available in one of the primary research sites, the National Maternity Hospital. MRI images included conventional T1/T2 weighted images, diffusion weighted images and proton magnetic resonance spectroscopy and they were reviewed and scored by an experienced consultant paediatric radiologist who was blinded to infant outcome (AB or GC). Barkovich,[32] NICHD[33] and de Vries[34] MRI scoring systems were applied to all available MRI images, however Barkovich scoring system was used for the definition of adverse outcome in this study as it provides excellent prognostic value for later neurodevelopmental outcome[35] and it is the most widely used and accepted scoring system at present for infants with NE.[36] The Barkovich scoring system examines the basal ganglia or thalamus (BG/T), applying a score of 0–4 and watershed (WS) areas, applying a score of 0–5. These scores are combined to provide a summary score from 0, indicating normal MRI, to 9, indicating the most extensive injury on MRI. Abnormal MRI brain was classified according to Barkovich scoring system. A score ≥1 indicated an abnormal MRI brain.

2.5 | Statistics

All data was assessed for normality of distribution by visual inspection of histograms and by Kolmogorov–Smirnov. If data was not normally distributed, a log transformation was applied or non-parametric tests were employed. Infants were categorised by outcome and group differences in time to SWC or SQ score between those with and without the primary outcome were examined. Group differences were examined using the Mann–Whitney U test. Correlation between time of onset of SWC or SQ scores, and MRI scoring systems were examined by Spearman's correlation coefficient (r_s). All tests were two-tailed, and the level of significance was set at $p<0.05$. Receiver operating characteristic (ROC) curves were used to compare the discriminatory ability of time to SWC and SQ to predict adverse outcome in NE, and the sensitivity, specificity, positive predictive value and negative predictive value were calculated. The interobserver agreement between the assessors of time to SWC and SQ score was measured by a non-parametric test, the Kendall's concordance coefficient (W). Correlations between different scoring systems were examined using Spearman's correlation coefficient. Statistical analysis was completed using SPSS (IBM SPSS statistics version 27).

2.6 | Ethical approval

This research was conducted as part of the NIMBUS and NEPTuNE studies which were approved by the local research ethics committees. Parents or guardians were provided with verbal information and a written patient information leaflet prior to consent for and enrolment in the research study. This research was funded by the National Children's Research Centre Ireland and the Health Research Board Ireland.

3 | RESULTS

There were 44 infants included in this study. All infants had continuous brainwave activity measured by aEEG recording for >60 h during TH as a routine part of their clinical treatment. Full outcome assessment was not possible for five infants due to missing MRI data. Two infants died prior to MRI assessment. Twenty one of the remaining 37 infants (57%) had abnormal MRI brain reported and 16 of 37 infants (43%) had normal MRI brain reports.

aEEG recordings were available for all 44 included infants. Mean duration of time from birth to onset of aEEG recording was 4.2 h. SWC was detected in the recording of 43 of 44 included infants (98%). SQ score could be calculated for all included infants and ranged from 0 to 13. All but one infant achieved SWC during the time of recording. The population median time to SWC was 13.5 h and the interquartile range (IQR) was 4 to 37.5 h. The population median SQ score was 12 and the IQR was 9 to 13. Results of duration to onset of SWC or and SQ scores for included infants with NE were not normally distributed, and this was confirmed by Kolmogorov–Smirnov tests.

MRI results were available for 37 of the 44 included infants (84%). 16 of the 37 (43%) infants had normal MRI brain, Barkovich score 0 and 21 of the 37 (57%) had abnormal MRI brain, Barkovich score ≥1. Therefore, 23 of the 39 (59%) infants had the primary definition of adverse outcome for this study. The median Barkovich score was 2 and the IQR was 0 to 4. Of the three infants who died in this study, only one had MRI brain and it was reported as demonstrating abnormalities. MRI Brain Barkovich scores were not normally distributed.

3.1 | Comparison of differences in time to SWC and SQ between groups

There was a statistically significant difference in time to SWC between groups of infants with normal and adverse outcome (Figure 1A), with infants that died or had abnormal MRI brain taking longer to achieve SWC than those that survived with normal MRI brain. For infants that survived with normal MRI brain the median time to SWC was 6 h and the IQR was 3-18 h. For infants that died or had abnormal MRI brain the median time to SWC was 23 h and the IQR was 13–55 h.

There were statistically significant differences in SQ score between groups, $p=0.002$ (Figure 1B), with lower SQ score in those infants who died or had abnormal MRI brain than those that survived with normal MRI brain. Infants with NE and survival with normal MRI brain the median SQ score was 13 (IQR 11.5–13; $p<0.05$)

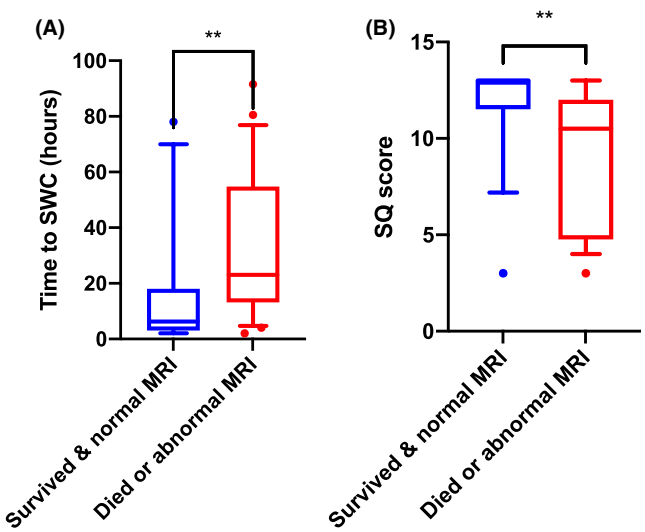

FIGURE 1 Differences in time to sleep wake cycling (SWC) and sleep quality (SQ) in infants with neonatal encephalopathy and good outcome (survived & normal MRI) or adverse outcome (died or abnormal MRI) ($n=44$). Differences between groups were significant in time to onset of SWC ($p=0.009$) (A) with infants with adverse outcome taking longer to achieve SWC. Differences between groups were significant in SQ score ($p=0.002$) (B) with infants with adverse outcome having lower SQ score.

compared to those who died or had abnormal MRI brain with a score of 10.5 (IQR 4.75–12; $p < 0.05$).

3.2 | Correlation of time to SWC and MRI scoring systems

Statistically, there was a significant positive correlation between longer time to onset of SWC and higher Barkovich (Figure 2A), NICHD (Figure 2B) and de Vries scores (Figure 2C). These all indicated that longer time to onset of SWC is correlated with greater abnormalities on MRI brain in NE. Therefore, although these findings suggest that there may be an overall relationship between delayed onset of SWC and worse MRI Barkovich score, these findings are not reliable and should be interpreted with great caution.

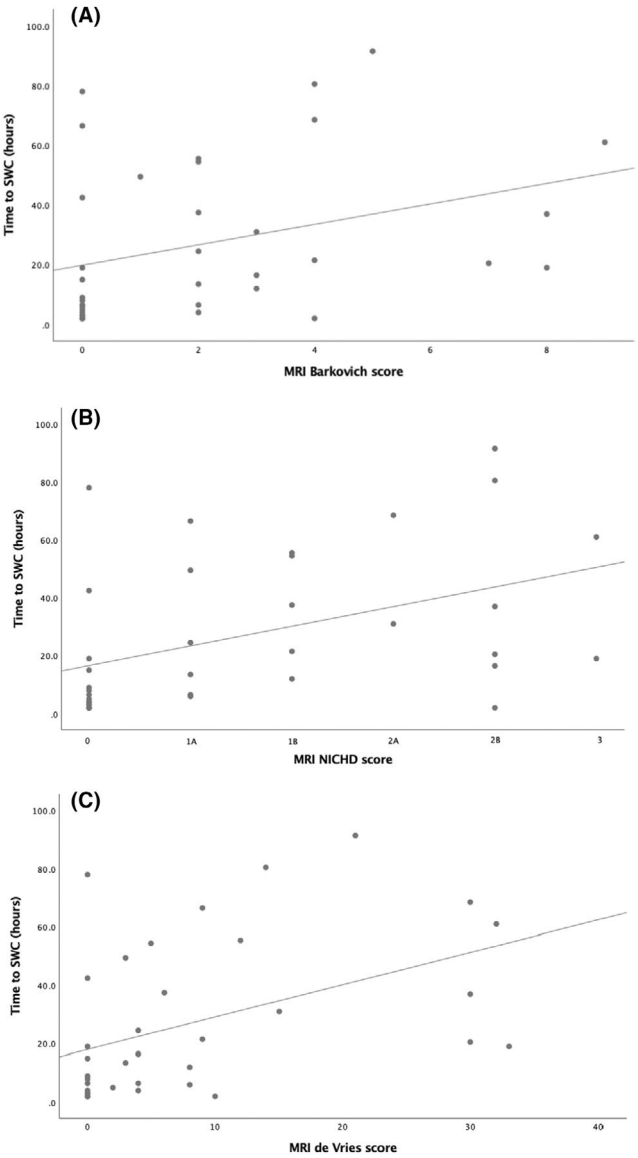

FIGURE 2 Correlation between time to onset of sleep wake cycling (SWC) and MRI scoring systems in infants with neonatal encephalopathy. There is a significant positive correlation between longer time to onset of SWC and higher Barkovich score ($p = 0.005$, $r_s = 0.454$) (A), NICHD ($p = 0.001$, $r_s = 0.526$) (B) and de Vries scores ($p = 0.001$, $r_s = 0.536$) (C).

3.3 | Correlation of SQ with MRI scoring systems

There was a significant negative correlation between lower SQ score and higher Barkovich (Figure 3A), NICHD (Figure 3B) and de Vries scores (Figure 3C). These all indicated that lower SQ score is correlated with greater abnormalities on MRI brain in NE.

3.4 | Comparison of discriminatory ability of time to SWC and SQ score to predict adverse outcome in neonatal encephalopathy

The calculated c-index for longer duration to SWC to predict adverse outcome in NE was 0.74 (95% CI 0.57 to 0.91). A time of 7.25 h to SWC gave a sensitivity of 76% and a specificity of 67% to predict adverse outcome in NE. A time of 11 h to SWC gave a sensitivity of 67% and a specificity of 80%.

The calculated c-index for higher SQ score to predict adverse outcome in NE was 0.84 (95% CI 0.71 to 0.97). A SQ score of 11.5 gave a sensitivity of 77% and a specificity of 80% to predict adverse outcome in NE. A SQ score of 12.5 gave a sensitivity of 91% and a specificity of 60%.

Compared to time to onset of SWC, sleep quality (SQ) as measured by Burdjalov scoring system provided higher sensitivity (77% vs. 67%), specificity (80% vs. 73%) to predict adverse outcome in NE and higher interobserver agreement (W 0.92 vs. $W = 0.86$) (Table 1).

3.5 | Interobserver agreement of time to sleep wake cycling and sleep quality score

As previously demonstrated the collected data for the predictor variables was not normally distributed. Therefore, the interobserver agreement between the assessors of time to SWC and SQ score was measured by the non-parametric test, Kendall's concordance coefficient (W). The interobserver agreement for time to SWC demonstrated $W = 0.86$, and there was statistically significant agreement between observers. The interobserver agreement for SQ score demonstrated $W = 0.92$, and there was statistically significant agreement between observers. Therefore, both measurements demonstrated good levels of interobserver agreement, with slightly better agreement for SQ scores.

3.6 | Association with longer duration to SWC and worse sleep quality with inflammatory cytokines

As disrupted sleep is associated with both recovery from inflammatory insults and worse outcome in NE, we examined the association between

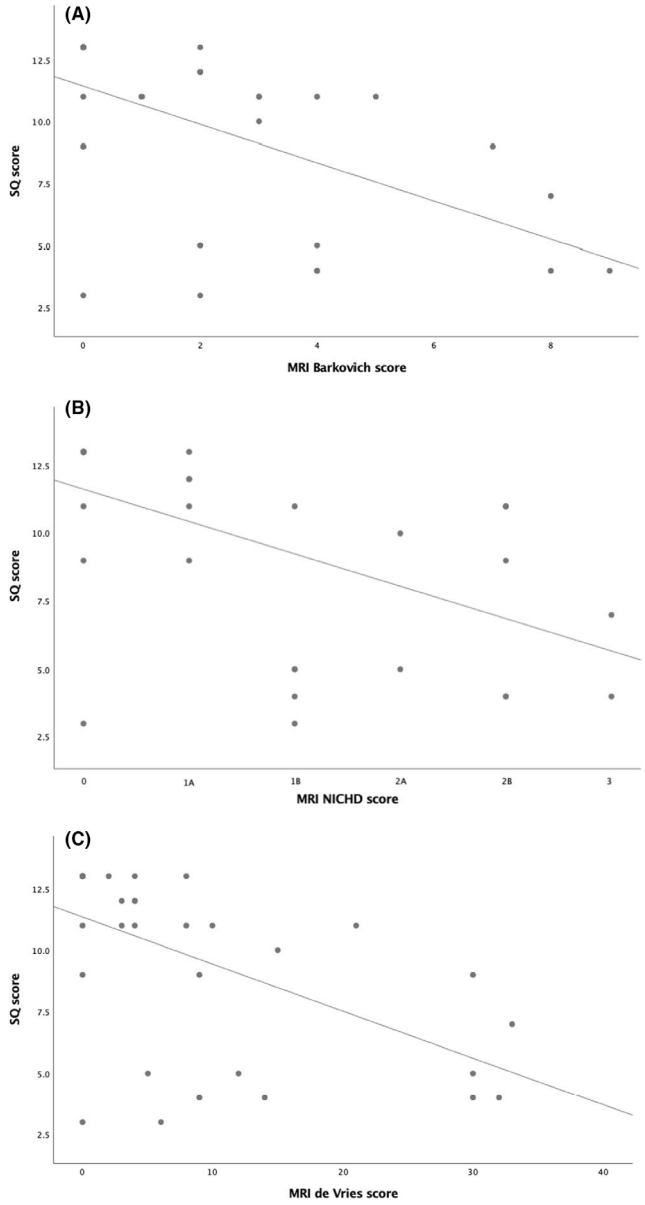

FIGURE 3 Correlation between time to onset of sleep quality (SQ) and MRI scoring systems in infants with neonatal encephalopathy. There was a significant negative correlation between lower SQ score and higher Barkovich ($p = <0.001$, $r_s = -0.642$) (A), NICHD ($p = <0.001$, $r_s = -0.688$) (B) and de Vries scores ($p = <0.001$, $r_s = -0.658$) (C).

time to SWC and SQ score and inflammatory cytokines in infants with NE. As the predictor and outcome data was not normally distributed, the non-parametric Spearman's correlation was used to examine the time to SWC and SQ scores and serum cytokine concentrations.

No significant correlations were found between time to onset of SWC and serum cytokine concentrations in infants undergoing TH for NE ($n = 37$; Table 2A). However, in infants with unfavourable outcomes ($n = 21$) a longer duration to SWC was associated with higher EPO and IL-8 infants undergoing TH for NE (Table 2B).

Lower SQ score was correlated with higher EPO and IL-1β ($n = 37$; Table 3A). However, in infants with unfavourable outcome ($n = 21$) lower SQ score was associated with higher TNF-α in infants undergoing TH for NE (Table 3B).

TABLE 1 Comparison of the discriminatory ability of time to sleep wake cycling (SWC) and sleep quality (SQ) score to predict outcome in infants with neonatal encephalopathy.

	Time to SWC	SQ Score
Sensitivity	67%	77%
Specificity	73%	80%
Positive PV	78%	85%
Negative PV	61%	71%
Kendall's W	0.86 ($p = 0.002$)	0.92 ($p < 0.001$)

Note: Compared to time to onset of SWC, SQ score provided higher sensitivity, specificity, negative and positive predictive value for adverse outcome in patients with NE and higher interobserver agreement.

4 | DISCUSSION

Infants with NE and adverse outcome had longer duration to onset of SWC and worse SQ. Longer duration to SWC has been associated with adverse outcome in previously reported studies.[2,30] The definition of time of onset to SWC by Takenouchi has previously been demonstrated to provide valuable prognostic information for infants with NE who are undergoing TH.[30] In a recent study reported the best overall prediction of outcome in NE with a combination of MRI and SWC on aEEG.[37] We found further evidence that time to SWC remains a good predictor of outcome in NE in the era of therapeutic hypothermia. We found a significant association between SQ, as measured by Burdjalov score,[31] and outcome in NE. This SQ score has previously been demonstrated to be associated with improved cerebral maturity and SQ in preterm infants[31] but to our knowledge it had not previously been applied to infants with NE. SQ score provided greater sensitivity and specificity to predict adverse outcome in NE compared to time to SWC. The evidence presented suggests that SQ score provides improved discriminatory ability to predict adverse outcome in NE when compared to time to SWC. However, the very wide confidence intervals for the c-index for both time to SWC and SQ, and the irregularity of the ROC curves, including some overlap beyond the line of random chance, demonstrate that the sample size is inadequate to draw any definitive conclusions. The evidence is to date is suggestive, but it is presumptive to draw this definitive conclusion based on the evidence currently available, as the sample size is too small and the evidence is not sufficiently robust. To reliably answer this question requires the same methodology to be repeated with a larger sample size. There was greater interobserver agreement for SQ score than time to SWC. However, the discriminatory ability of either SQ or SWC to predict outcome in NE was still inferior to MRI,[36] but requires less resources and provides earlier prognostic information than MRI.

Better SQ was negatively associated with serum EPO and IL-1β concentrations, and that in patients with unfavourable outcomes

TABLE 2 Correlation between time to sleep wake cycling (SWC) and serum cytokines in all infants with neonatal encephalopathy (A), and correlation between SWC and serum cytokines in infants with unfavourable outcome following neonatal encephalopathy (B), as measured by Spearman correlation coefficient. In infants with unfavourable outcomes a longer duration to SWC was positively associated with serum EPO and Interleukin (IL)-8.

Cytokine	Spearman (*r*)	*p*-value
A		
EPO	0.20	0.27
GMCSF	0.32	0.86
IFN-γ	0.19	0.31
IL-1α	0.12	0.58
IL-1ra	0.12	0.95
IL-1β	−0.01	0.94
IL-6	0.05	0.77
IL-8	0.05	0.74
IL-10	0.00	0.99
IL-18	0.09	0.61
TNF-α	0.12	0.51
TNF-β	0.08	0.71
VEGF	0.17	0.35
B		
EPO*	0.63	0.03
GMCSF	0.14	0.66
IFN-γ	−0.03	0.93
IL-1α	0.17	0.67
IL-1ra	0.43	0.16
IL-1β	0.09	0.77
IL-6	0.42	0.13
IL-8*	0.56	0.03
IL-10	0.32	0.27
IL-18	0.28	0.38
TNF-α	0.35	0.22
TNF-β	0.66	0.08
VEGF	0.48	0.10

*Indicates a significant correlation with the level of significance set at $p < 0.05$.

TABLE 3 Correlation between sleep quality score (SQ) and serum cytokines in all infants with neonatal encephalopathy (NE) (A), and correlation between SQ and serum cytokines in infants with unfavourable outcome following neonatal encephalopathy (B), as measured by Spearman correlation coefficient. In infants with NE undergoing therapeutic hypothermia (TH) higher SQ was negatively associated with serum EPO and interleukin (IL)-1β. In infants with unfavourable outcomes higher SQ was negatively associated with serum tumour necrosis factor (TNF)- α.

Cytokine	Spearman (*r*)	*p*-value
A		
EPO*	−0.42	0.02
GMCSF	−0.14	0.44
IFN-γ	−0.32	0.07
IL-1α	−0.23	0.27
IL-1ra	−0.19	0.28
IL-1β*	−0.35	0.04
IL-6	−0.14	0.91
IL-8	−0.20	0.06
IL-10	−0.25	0.15
IL-18	−0.24	0.19
TNF-α	−0.25	0.16
TNF-β	0.19	0.34
VEGF	−0.08	0.68
B		
EPO	−0.49	0.10
GMCSF	−0.20	0.52
IFN-γ	−0.21	0.49
IL-1α	−0.41	0.28
IL-1ra	−0.51	0.09
IL-1β	−0.39	0.19
IL-6	−0.34	0.24
IL-8	−0.51	0.06
IL-10	−0.53	0.05
IL-18	−0.53	0.08
TNF-α*	−0.62	0.02
TNF-β	−0.41	0.32
VEGF	−0.51	0.07

*Indicates a significant correlation with the level of significance set at $p < 0.05$.

shorter duration to onset of SWC and is associated with higher EPO concentrations and that higher SQ was associated with lower TNF-α concentrations. There is evidence of a bidirectional relationship between disrupted sleep and dysregulated inflammation,[1] including alterations in cytokine production.[38] It remains unclear whether sleep disruption is a consequence of worse brain injury following NE or whether sleep may play a causal role in worsening dysregulated inflammation and outcome in NE. However, there is biological plausibility that disrupted sleep may play a causal role and this requires further investigation. Sleep disruption has been associated with increased EPO concentrations in other infant cohorts[39] and although EPO was proposed as a treatment for infants with NE, a recent large trial of EPO administration was associated with increased risk of adverse outcome in NE.[40]

Both IL-1β and TNF-α promote sleep, particularly NREM sleep,[41] and are associated with adverse outcome in NE.[42] Therefore, it is not surprising that an association was found in this study, and that their concentrations showed an inverse relationship to SQ. It remains unclear however, in which direction this relationship occurs. Several factors associated with sleep onset are disrupted during care in NICU, including excessive light exposure,[43] excessive noise exposure[44] and disruption to feeding patterns.[17] These factors are

modifiable. Although no study has demonstrated any intervention to improve sleep in NICU,[45] light–dark cycling has demonstrated improved outcomes in infants born prematurely.[24] With a sufficient sample size, it may be possible to statistically control for other early prognostic biomarkers and better isolate the association between sleep and outcomes in NE. A limitation of the study is the lack of measurement of serial cortisol in relation to circadian rhythms due to inadequate remaining samples and access to metabolomic expertise.[46]

Our intention was to control for the severity of NE for a more direct examination of the relationship between worse sleep states and dysregulated inflammation or adverse outcome. The purpose was to assess more specifically if adverse sleep may be a contributing factor to dysregulated inflammation and adverse outcome. The best measure to assess the severity of NE in the first few days is standardised clinical exam and Sarnat staging. Unfortunately, there were insufficient numbers of infants in each group to control for severity of NE. Questions remain whether longer duration to onset of SWC or worse SQ reflects worse brain injury or whether circumstances that interfere with sleep onset and quality may exacerbate dysregulated inflammation and lead to worse outcomes in NE. Sleep disruption persists in NE[47] and early interventions to improve it may provide improved long-term outcomes.[14]

AUTHOR CONTRIBUTIONS

Tim Hurley: Conceptualization; investigation; writing – original draft; methodology; writing – review and editing; data curation; software. **Philip Stewart:** Investigation; data curation. **Robert McCarthy:** Writing – review and editing. **Mary O'Dea:** Data curation; conceptualization; investigation; methodology; visualization. **Lynne Kelly:** Formal analysis; data curation; resources. **Mandy Daly:** Project administration; data curation; resources; software. **John Butler:** Resources; data curation; project administration; formal analysis. **Rob McCarthy:** Writing – review and editing; writing – review and editing. **Jan Miletin:** Resources; formal analysis; data curation. **Deirdre Sweetman:** Resources; formal analysis. **Angela Byrne:** Formal analysis. **Gabrielle Colleran:** Formal analysis. **Megan Ni Bhroin:** Formal analysis. **Arun L. W. Bokde:** Formal analysis. **Eleanor J. Molloy:** Funding acquisition; conceptualization; methodology; visualization; project administration; supervision; resources; writing – review and editing; investigation; data curation.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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