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Total Daily Production and Periodicity of Melatonin Metabolite in Critically Ill Children

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Objectives: To determine whether total daily 6-sulfatoxymelatonin excretion and diurnal variation of melatonin secretion was maintained during the early phase of PICU admission through examination of the melatonin urinary metabolite, 6-sulfatoxymelatonin.

Design: Exploratory prospective, observational study.

Setting: Twelve-bed medical-surgical PICU of a Children's Hospital.

Patients: Fifty children 3 months to 18 years old enrolled within 24 hours of PICU admission with access for urinary sampling.

Interventions: None.

Measurements and Main Results: Urine samples were collected at 4-hour intervals for 24 hours and stored at −80°C. 6-sulfatoxymelatonin was determined in duplicate by direct enzyme-linked immunosorbent assay. Patients were heterogeneous for diagnosis, had a mean age of 8.1 years (SD = 6.1 yr), and median (interquartile range) Pediatric Risk of Mortality III of 10 (4–13). Mean (SD) total daily 6-sulfatoxymelatonin production was 30.0 µg (25.6 µg) for the first 24 hours, which did not differ significantly from the means on days 2 ($p = 0.56$) or 3 ($p = 0.29$), and was similar to literature controls. Mean 6-sulfatoxymelatonin production for the population fit a periodic function well, with a reliable amplitude of 326 ng/hr and peak excretion from 04:00 to 08:00 ($F = 4.4$, $p = 0.01$), even when 6-sulfatoxymelatonin was corrected for body weight ($F = 3.4$, $p = 0.03$) and when sedation was included in the model ($F = 3.95$, $p = 0.004$). There was no significant correlation between lighting and 6-sulfatoxymelatonin excretion at any time period (R^2 values: 0.11–0.25, $p = 0.10$–0.94). Mean 6-sulfatoxymelatonin excretion did not fit the model for a periodic function well for the subpopulations studied (sepsis [$n = 18$, $F = 1.1$, $p = 0.32$], respiratory failure requiring deep sedation [$n = 10$, $F = 0.4$, $p = 0.66$], and neurologic injury [$n = 7$, $F = 0.6$, $p = 0.55$]).

Conclusions: Total daily and diurnal variation of 6-sulfatoxymelatonin excretion is heterogeneously maintained early in pediatric critical illness. However, this may not hold true for specific diagnostic categories. (*Pediatr Crit Care Med* 2020; XX:00–00)

Key Words: 6-sulfatoxymelatonin; circadian rhythm; critical illness; melatonin; pediatrics; periodicity

Critically ill children are likely to experience poor sleep quality, abnormal sleep-wake cycles, and delirium (1). Although some sleep disturbances may be unavoidable during an admission to a PICU, efforts aimed at protection of sleep and maintenance of normal sleep cycles may result in improved comfort, decreased need for sedatives, decreased delirium, and improved healing (2). Melatonin impacts sleep as it is an important endogenous hormone in human circadian rhythm generation. To this end, an improved understanding of melatonin production in critically ill children may offer opportunities for preventative and therapeutic strategies.

Melatonin is produced and secreted by the pineal gland. The major product of melatonin metabolism is 6-sulfatoxymelatonin (aMT6s), which is produced in the liver and renally excreted in concentrations that correlate with serum melatonin concentration (3, 4). Peak nocturnal serum melatonin concentrations tend to vary with age, with a peak between 1 and 3 years old and subsequently decreasing proportional to body weight until adulthood (5). However, circadian rhythmicity including timing of peak serum melatonin and day-night variation in aMT6s excretion, total pineal melatonin secretion, and total daily excretion of aMT6s (TD aMT6s) is stable through early childhood and adolescence (6–9).

Pineal function in critical illness and within the intensive care environment is not well understood. Pineal gland melatonin production is triggered by lack of light; it generally increases between 21:00 and 23:00, peaks between 01:00 and 04:00, and returns to baseline in the morning from 06:00 to

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DOI: 10.1097/PCC.0000000000002461

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09:00 (10). Diurnal variation of melatonin secretion may be affected by several factors common to PICU admissions including stress-induced endogenous catecholamines, exogenous adrenergic agents, opioids, benzodiazepines, corticosteroids, and a lack of normal lighting cues (11–16). Critically ill adults demonstrate disturbed diurnal rhythm of melatonin secretion with loss of periodicity in several contexts (11, 17–20). Two recent case series of melatonin secretion in critically ill children indicated altered peak serum timing and concentrations compared with literature controls, with unchanged total daily production of both melatonin and aMT6s (21–23). However, the rhythmicity of melatonin secretion is difficult to characterize as peak serum levels exhibit significant inter-individual variation in both timing and concentration (4, 24), and complete characterization would require an unacceptable number of blood draws over the course of a pediatric illness.

Therefore, we performed an exploratory, hypothesis-generating prospective observational study to: 1) determine whether melatonin secretion periodicity was maintained in critically ill pediatric patients early in their admission (within the first 3 d) by examining aMT6s excretion; 2) compare TD aMT6s with literature controls; and 3) examine TD aMT6s and aMT6s periodicity in specific diagnostic subpopulations (sepsis, respiratory failure requiring sedation, and neurologic injury). Based on the previous series (21–23), we hypothesized that diurnal variation of melatonin secretion is lost in children during critical illness, whereas TD aMT6s is maintained.

MATERIALS AND METHODS

This study was approved by the Western University Health Sciences Ethics Board and the Lawson Research Quality and Compliance Committee. Our PICU is a 12-bed medical/surgical unit that admits more than 600 critically ill/injured patients annually, 0–17 years old, and serves a catchment area of 190,000 square kilometers with a pediatric population of greater than 50,000.

Study Design and Participants

The study was designed to be exploratory, as there was no guiding literature on melatonin excretion in critically ill children at study inception. Patient screening was performed over a 4-year period on a convenience basis when the primary investigator was available and enrollment proceeded for eligible patients when a laboratory assistant was available for proper sample transport, handling, and storage. Patients or their guardians were approached for consent within the first 24 hours of admission if they: 1) were admitted to the PICU with a Pediatric Risk of Mortality (PRISM) III greater than or equal to 1; 2) were between 52 weeks postconceptual age (onset of circadian rhythm after 12 wk old [3]) and 17.9 years old; 3) had either an indwelling urinary catheter or the ability to urinate on a commode or toilet to allow complete urine collection; and 4) were expected to remain in the PICU for at least 24 hours after the start of sample collection. Patients were excluded if they: 1) were normally on oral melatonin supplementation or a β-blocking agent (may impair melatonin production); 2) had a history of sleep-wake cycle abnormality at baseline; 3) were receiving continuous renal replacement therapy; or 4) had a history of depression, insomnia, thyroid disorder, severe substance abuse or dependency on nicotine or alcohol, liver failure, cirrhosis, or liver transplant within the previous month. Patient diagnosis was confirmed by comparison of admission and discharge diagnoses, and review of relevant investigations to determine the actual cause of critical illness. Sepsis and septic shock were defined using consensus conference criteria (25). Respiratory failure was defined as need for mechanical ventilation from a primary or secondary pulmonary cause that results in oxygenation or ventilation failure, although not intubation and/or mechanical ventilation for respiratory drive, minimization of energy consumption, or airway protection. Deep sedation was defined as a median State Behavioral Scale (SBS) (26) score of –2 to –3 or, when SBS was unavailable, a sedative medication score (SMS) (summarized below) of at least 10 for greater than 50% of the day. Neurologic injury was defined as blunt, ischemic, or surgical injury to brain parenchyma.

Patient Care

All patient care was at the discretion of the treating team. In particular, unit practice was for the treating team to set daily patient-specific sedation (SBS) and analgesia (Faces, Legs, Activity, Cry, Consolability Observational Tool-revised) targets, which were assessed by nursing staff every 4 hours. Infusions of midazolam and morphine were standard. We recorded all sedative and analgesic agents, converted benzodiazepines to midazolam and opioids to morphine equivalents, and then calculated a summary sedative score (the SMS), both using the method described by Randolph et al (27), modified to include ketamine (28) and clonidine. This score assigns a value of 1 point to the amount of sedation that would be estimated to sedate a nontolerant individual for 1 hour using the following formula: sedation score = opioid points (mg/kg morphine equivalents/0.1) + benzodiazepine points (mg/kg midazolam equivalents/0.1 + chloral hydrate points (mg/kg/50) + ketamine points (mg/kg/2) + clonidine points (μg/kg/5) + propofol points (1/hr used) + phenobarbital points (1/hr used) + antihistamine points (0.5/d of use). Vasoactive infusions were recorded and converted to the pediatric Vasoactive-Inotropic Score (29).

We examined nursing documentation from the time of sedative weaning until 2 days after discharge from PICU for evidence of sleep-wake cycle abnormalities. We considered frequent awakenings, inadequate nighttime or excessive daytime sleep, or subjective nurse description of sleep cycle abnormalities as evidence of a sleep-wake cycle abnormality.

Lighting and Sound Management

Lighting and noise level were measured every 4 hours at the patient's eye-level using a multifunction meter with sound and light measurement (Multifunction Lux Meter PCE-EM 882, PCE Instruments, Jupiter, FL). An order was placed in each patient chart for lights to be on in the day (at least 10:00 to 18:00) and off at night (at least 22:00 to 06:00), although there were no interventions to ensure controlled lighting or noise conditions. Normal day/night light patterns were considered dim

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light (< 50 lux) at night and light levels sufficient to suppress melatonin production (> 100 lux) (30) during the day.

Urine Sampling and Analyses

Urine was collected from an indwelling urinary catheter every 4 hours at 02:00, 06:00, 10:00, 14:00, 18:00, and 22:00. Urine was emptied from the collection bag at the earliest time point after consent was obtained. Thereafter, urine volume was recorded every 4 hours, whereby a sample of 5–10 mL of urine was collected in a clean specimen container, and the remaining urine for that period was discarded. For spontaneously voiding children, the time of the last void was noted; for all subsequent voids, the volume and time was noted and a 5–10 mL sample was retained. Urine was transported within 24 hours of collection (6) to a storage facility, where it was stored at -80°C.

Urine samples were collected for 24 hours from the start of urinary collection until urine collection was no longer possible due to Foley removal or if the child was discharged from the PICU. In some cases, urine collection occurred beyond the 24-hour standard to determine melatonin secretion patterns beyond the acute phase of the illness. In these cases, melatonin secretion was longitudinally measured by collecting urine on days 1, 2, 3, 7, 10, and 14, and ending early for PICU discharge.

Urinary aMT6s was assayed in duplicate following the instructions provided by the commercially available enzyme-linked immunosorbent assay (ELISA) kit (catalog number RE54031; IBL, Hamburg, Germany) that had the following characteristics: sensitivity 1.0 ng/mL; intra-assay coefficient of variation (CV) 5.2–12.2%; and inter-assay CV 5.1–14.9%.

Data Analysis

Total aMT6s for each collection period was calculated as measured concentration multiplied by the urinary volume for the period. The aMT6s excretion (ng/hr) was calculated as the total aMT6s for the collection divided by the duration of the collection period. TD aMT6s excretion (ng) was calculated for patients who had a full 24-hour collection. Nocturnal melatonin production was assumed to be represented by the urinary collections at 02:00, 06:00, and 10:00, reflecting serum melatonin from approximately 20:00 to 08:00.

Data were analyzed using SPSS Version 25.0 (IBM Corp., Armonk, NY) and R Version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org). Categorical data were reported as percentages. Continuous normative data were reported as means ± SDs; otherwise, nonparametric data were reported as medians with interquartile ranges (IQRs). A literature-based reference population of healthy outpatient children from the Dortmund Nutritional and Anthropometric Longitudinally Designed Study was used for comparison of aMT6s mean values. Each subject in this longitudinal study (n = 66) had between three and 15 measurements of total daily aMT6s, which was measured using ELISA (31). Independent samples t tests were used to compare aMT6s mean values with literature controls. Cosinor analyses were conducted using the "cosinor" package in R (retrieved from http://github.com/sachsmc/cosinor). This analysis used the least squares method to fit the sine wave of the longitudinal aMT6s data to a 24-hour series and tested the reliability of the intercept and amplitude values. Linear regression was used to examine overall model fit for periodic function. For patients with more than one day of data, a repeated measures analysis of variance was used to test periodicity by examining the interaction between time and day on aMT6s production, with a nonsignificant interaction ($p > 0.05$) indicating that periodicity was upheld. We included lighting in the regression as a dichotomous variable, where day-night lighting variation was either normal (> 100 lux in the day and < 50 lux at night) or not. We included sedation level (SBS) and sedation medication score in the regression as continuous variables. Diurnal variation was examined by comparing total nocturnal and daytime aMT6s excretion using the Wilcoxon signed-rank test.

RESULTS

We enrolled a total of 50 critically ill patients over 4 years. Enrollment was predominantly hindered by availability of research personnel and requirements for a reliable method for collecting all urine for at least 24 hours. Two families did not consent. Forty-seven patients completed a full 24-hour collection; three ended early for catheter removal, early discharge, and transfer to operating room during collection. Three samples were either lost or insufficient, resulting in 44 patients with complete 24-hour urine collections. Ten patients had samples collected for more than 24 hours, five patients for more than 48 hours, and two for more than 72 hours.

The sample was heterogeneous, with mean (SD) age of 8.1 years (6.1 yr), median (IQR) weight of 23.5 kg (12.8–53.8 kg), and median (IQR) PRISM III of 10 (4, 13). Baseline characteristics are given in **Table 1**. Although most patients received mechanical ventilation (88%), other therapies were heterogeneous (Table 1). After sedating agents had been weaned and sleep could be observed, seven patients (14%) had sleep-wake cycle abnormalities documented.

Mean TD aMT6s excretion in the first 24 hours of measurement was 30.0 µg (SD = 25.6 µg), which did not differ significantly from the means on days 2 ($p = 0.56$) or 3 ($p = 0.29$; **Table 2**). Literature-based healthy comparison data measured using ELISA were not available for aMT6s excretion for children less than 3 years old. However, mean total aMT6s excretion for patients 3–17.9 years old on days 1 and 2 was similar to those reported previously of 38.6 µg (SD = 15.9 µg) ($n = 66$) (31) (Table 2). The mean aMT6s in our sample population was significantly lower for patients 3 months to 3 years old at 13.6 ng (SD = 9.8 ng), compared with those 3–17.9 years old at 37.7 ng (SD = 27.0 ng) ($p < 0.01$).

For our entire sample population, the mean aMT6s excretion on day 1 fit a 24-hour cosine model well (**Fig. 1**; F = 4.43, $p = 0.01$), even when aMT6s was corrected for body weight (F = 3.4, $p = 0.03$). Peak excretion was at 08:00, corresponding to peak serum levels between 04:00 and 07:00 (32), and the amplitude of the function was 326 ng/hr ($p < 0.01$). Excretion of aMT6s for 10 patients on day 2 also fit a 24-hour cosine

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TABLE 1. Admission and Demographic Data and Treatment Characteristics

Patient Characteristics	All Patients (*n* = 50)
Male sex, *n* (%)	34 (68)
Age, yr, mean (SD)	8.1 (6.1)
Weight, kg, median (IQR)	23.5 (12.8–53.8)
Pediatric Risk of Mortality III, mean (SD)	10.7 (7.1)
Sepsis (*n* = 18)	11.3 (5.8)
Respiratory failure sedated (*n* = 10)	8.2 (4.5)
Neurologic injury (*n* = 7)	14.7 (8.3)
Chronic diagnosis, *n* (%)	22 (44)
Atopy	9 (18)
Neurologic	3 (6)
Oncologic	5 (10)
Mental health	5 (10)
Admission diagnosis, *n* (%)	
Sepsis	18 (36)
Respiratory failure	12 (24)
Nonhead trauma	5 (10)
Traumatic brain injury	5 (10)
Other (intracranial pathology, infection nonseptic, oncologic, cardiac, overdose, status epilepticus)	10 (20)
Room with a window, *n* (%)	30 (60)
Acute kidney injury (elevated serum Cr > 2 × normal for age), *n* (%)	2 (4)
Acute liver failure (liver enzymes > 3 × normal for age), *n* (%)	3 (6)
Mild elevation of liver enzymes (> 3 × normal, < 1,000 U/L), *n* (%)	3 (6)
Mechanically ventilated, *n* (%)	
Invasive	40 (80)
Noninvasive	4 (8)
Vasoactive infusion score (23), median (IQR)	0 (0–3.75)
Sedative medication score (22), median (IQR)	34.5 (9.7–73.7)
Sepsis (*n* = 18)	4.5 (0.3–8.3)
Respiratory failure sedated (*n* = 10)	14.3 (8.8–20.2)
Neurologic injury (*n* = 7)	7.5 (5.0–12.8)
State Behavioral Scale (26), median (IQR)	−1 (−2 to 0)
Sepsis (*n* = 18)	−1 (−2 to 0)
Respiratory failure sedated (*n* = 10)	−1 (−2 to −1)
Neurologic injury (*n* = 7)	−2 (−3 to −2)
Muscle relaxant use, *n* (%)	14 (28)
Corticosteroid use, *n* (%)	24 (48)
Receiving feeds, *n* (%)	31 (62)
Abnormal day/night light variation, *n* (%)	37 (74)
Noise (dB), mean (SD)	
Day	48.5 (6.4)
Night	51.8 (8.8)

IQR = interquartile range.

model well (Table 2). The amplitude peak occurred between approximately 06:00 and 07:00 and did not demonstrate a significant phase shift. Numbers were insufficient to test fit on day 3. For patients with complete 48 hour data ($n = 7$), there was no interaction between time and day in terms of aMT6s hourly excretion, indicating that periodicity was upheld over 2 days ($p = 0.75$). There was no significant correlation between lighting and aMT6s excretion at any time period (R^2 values: 0.11–0.25; $p = 0.10$–0.94). When the patient's SMS and SBS were included in the model, periodicity was upheld ($F = 3.95$, $p = 0.004$).

We superimposed the aMT6s excretion from three distinct pathologies to determine if their excretion patterns were similar to the total group of patients (**Figs. 2–4**).

Sepsis

There were 18 patients with sepsis, of whom nine had septic shock (Fig. 2). A total of 14 patients were intubated and mechanically ventilated. As a group, patients with sepsis demonstrated a reliable amplitude (362.8 ng/hr; $p = 0.04$) with a peak of 1,806.4 ng/hr occurring between 07:00 and 08:00. However, the data did not fit the model well for periodic function ($F = 1.14$, $p = 0.32$; Table 2). No patients died; four septic patients (three with septic shock) had documented sleep-wake cycle abnormalities after lifting sedation.

Respiratory Failure

There were 10 patients with respiratory failure receiving invasive mechanical ventilation and deep sedation (Fig. 3), of whom one patient also fulfilled sepsis criteria. Cosinor analysis demonstrated poor fit to a 24-hour periodic function model ($F = 0.41$, $p = 0.66$; Table 2). One patient died in PICU and one had a documented sleep-wake cycle abnormality after extubation.

Neurologic Injury

Seven patients experienced neurologic injury, including traumatic brain injury, tumor, and intracranial bleeding, requiring intubation, and mechanical ventilation (Fig. 4). The data did not fit a periodic function well ($F = 0.60$, $p = 0.55$; Table 2). No

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TABLE 2. 6-Sulfatoxymelatonin Excretion Characteristics for Entire Group and Predefined Subgroups

Patient Population	24-hr aMT6s Excretion, Mean (SD), µg	Comparison to Literature Control[a], *p*	Cosinor Amplitude (ng/hr)	Amplitude Reliability, *p*	Cosinor Peak time	Analysis of Variance F, *p*
Day 1, total population ($n = 44$)	30.0 (25.6)	0.84	326	$p < 0.01$	08:00	$F = 4.41$, $p = 0.01$
Day 2, total population ($n = 7$)	23.4 (12.9)	0.56	613	$p < 0.0001$	07:00	$F = 4.78$, $p = 0.01$
Total population, day 3 ($n = 4$)	45.5 (47.9)	0.29	NA		NA	
Sepsis ($n = 18$)	44.2 (20.3)	0.14	362.8	0.04	08:00	$F = 1.14$, $p = 0.32$
Respiratory failure sedated ($n = 10$)	27.1 (8.1)	0.12	103.4	0.36	11:00	$F = 0.41$, $p = 0.66$
Neurologic injury ($n = 7$)	33.7 (43.0)	0.57	504.8	0.28	08:00	$F = 0.60$, $p = 0.55$

aMT6s = 6-sulfatoxymelatonin.

[a]Comparison to literature control, Griefahn et al (24) included only children in the correct age category (age 3–18 yr).

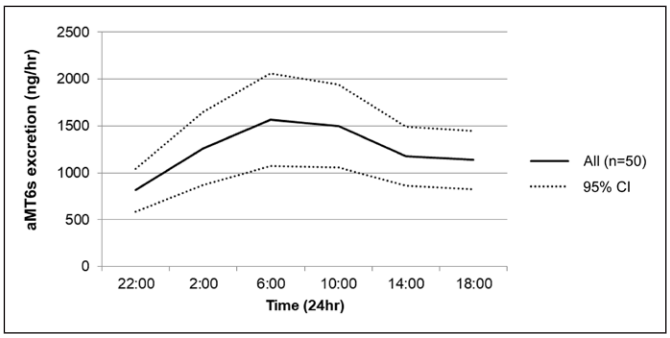

Figure 1. Twenty-four-hr 6-sulfatoxymelatonin (aMT6s) cosine model, total population. *y*-axis: aMT6s excretion (ng/hr) and *x*-axis: time (24 hr). All ($n = 50$) and 95% CI.

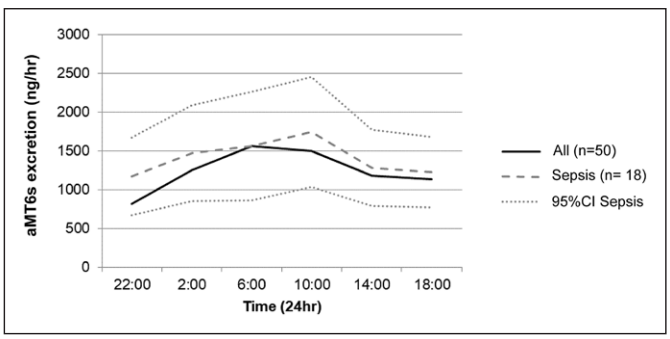

Figure 2. Twenty-four-hr 6-sulfatoxymelatonin (aMT6s) cosine model, sepsis. *y*-axis: aMT6s excretion (ng/hr) and *x*-axis: time (24 hr). All ($n = 50$), sepsis ($n = 18$), and 95% CI (sepsis).

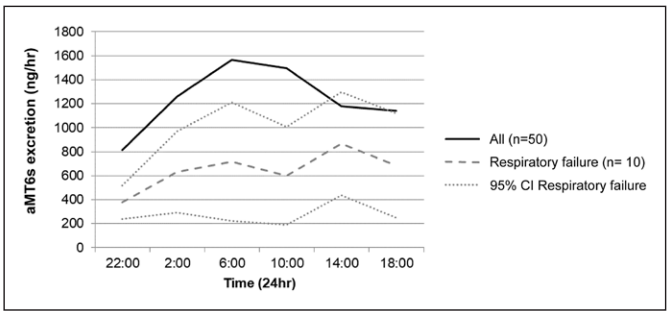

Figure 3. Twenty-four-hr 6-sulfatoxymelatonin (aMT6s) cosine model, respiratory failure. *y*-axis: aMT6s excretion (ng/hr) and *x*-axis: time (24 hr). All ($n = 50$), respiratory failure ($n = 10$), and 95% CI (respiratory failure).

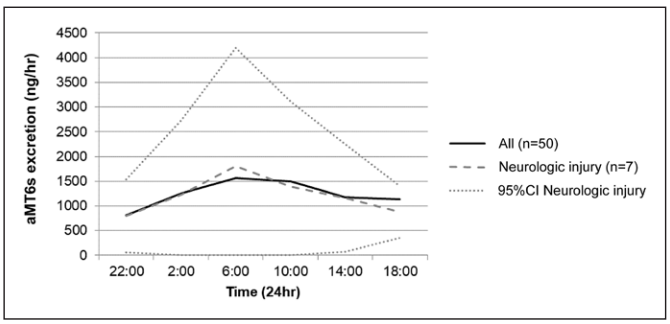

Figure 4. Twenty-four-hr 6-sulfatoxymelatonin (aMT6s) cosine model, neurologic injury. *y*-axis: aMT6s excretion (ng/hr) and *x*-axis: time (24 hr). All ($n = 50$), neurologic injury ($n = 7$), and 95% CI (neurologic injury).

surviving patients had documented postextubation sleep-wake cycle abnormalities.

Validity of the periodicity findings was tested by examining diurnal variation for patients who had paired, complete day and night samples. Diurnal variation was maintained for the population as a whole and for the septic subgroup (**Table 3**).

DISCUSSION

This study describes melatonin variation in critically ill children. In this cohort, examined early in their illnesses, the averaged values of both the total melatonin metabolite (aMT6s) excretion and variation in excretion throughout the day reflected normal patterns. However, closer analysis revealed heterogeneous behavior between individual patients and subgroups defined by clinical diagnosis. Our findings contradict previous adult and pediatric data, which had led us to hypothesize that the subgroups would be more homogeneous in their maintenance or loss of diurnal variation.

Only one other study has examined melatonin rhythmicity in critically ill children (23). Their examination of 16 analgosedated children over 14 hours demonstrated lack of a serum peak in two children, with phase shifting in a further 11 patients

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TABLE 3. Diurnal Variation for Cohort With Complete 24-Hour Sample Collection

Population	Daytime aMT6s (μg), Median (IQR)	Nocturnal aMT6s (μg), Median (IQR)	*p* (Wilcoxon Signed-Rank Test)
Total group ($n = 44$)	10.0 (5.4–14.8)	14.0 (64.7–22.8)	0.002
Sepsis ($n = 16$)	11.4 (6.5–16.6)	16.6 (11.9–28.9)	0.039
Respiratory failure sedated ($n = 10$)	7.0 (2.0–12.0)	5.8 (1.5–12.4)	0.96
Neurologic injury ($n = 6$)	9.4 (6.1–19.1)	7.8 (1.7–34.2)	0.75
Remainder of patients ($n = 13$)	10.7 (5.6–16.6)	18.2 (9.6–26.9)	0.006

aMT6s = 6-sulfatoxymelatonin, IQR = interquartile range.

(assuming normal serum peak between 01:00 and 03:00). Based on this criterion, our sample also demonstrated phase shifting, with peak aMT6s excretion at 08:00, corresponding to peak serum levels between 04:00 and 07:00. The low amplitude of averaged aMT6s excretion peaks in our study, along with the wide CIs at each time point, may be at least partially explained by significant inter-individual variation that is normally seen in the amplitude and timing of nocturnal serum melatonin peaks (33), thereby flattening the amplitude for the cohort.

We designed our study intending to explore individual populations that were homogeneous for diagnosis (sepsis, respiratory failure with deep analgosedation, and neurologic injury), as these are populations that have been specifically studied in the adult literature (11–14, 31, 32). Our a priori defined subgroups demonstrated visually obvious inter-individual heterogeneity in maintenance of periodicity whereas, in similarly composed adult populations studied within the first 3 days of illness, patients homogeneously lost normal diurnal variation early in their illness (17–19). Although we may speculate that this is be related to either different ICU practices (e.g., analgosedation) or to neurodevelopmental differences in pediatrics, the low patient numbers and differing study methodology do not allow for direct comparisons to any one subgroup. Interestingly, the sample as a whole and the septic subgroup maintained diurnal variation of aMT6s excretion, while it was not maintained in the patients with neurologic injury or those deeply sedated with respiratory failure. This suggests that centrally acting effects of either disease or medication may impact aMT6s periodicity, although we did not find a significant relationship between SBS or SMS and maintenance of periodicity in our small cohort. Further research would ideally include longitudinal examination of melatonin secretion for individual, homogeneous populations that enables differentiation between effects of the environment (e.g., light and sound), therapy (e.g., analgosedating agents and mechanical ventilation), and the illness.

In our exploration of melatonin rhythmicity, we continued the examination beyond a 24-hour period for 12 patients and for more than 3 days in two patients. This subgroup statistically maintained periodicity over day 2, and we noted visually that several patients with initially attenuated aMT6s excretion regained an early morning peak over several days. Future research with larger sample sizes is needed to corroborate these findings.

Our patients 3–17.9 years old maintained a mean TD aMT6s that was similar to literature-based controls (7, 31, 34, 35). TD aMT6s correlates well with the area under the curve for serum melatonin over 24 hours (4) and with peak plasma concentrations in adults (36). In addition, nocturnal aMT6s (from 22:00 to 07:00) correlates with 02:00 melatonin peaks (37), with the estimated lag from serum melatonin peak to aMT6s peak of 1–4 hours (4, 32). Our data were derived from urinary samples only, as serum melatonin concentrations tend to decrease with age, whereas TD aMT6s excretion does not. Measured aMT6s is variable depending on the measurement technique used (4, 6), and so we were cautious to compare our results only to studies that used similar measurement techniques. Although we were unable to compare the mean TD aMT6s for children 3 months to 3 years with literature controls, the mean was lower than in older children.(38) Although we speculate that this could be related to more tenuous circadian rhythms or pineal function in this age group, we did not demonstrate any consistent age-based relationships with mean TD aMT6s. The relationship between melatonin secretion during critical illness and pediatric patient age warrants further examination.

Others have found that TD aMT6s within the first 3 days of illness was similar between pediatric septic and nonseptic controls, and was lower than in both our septic cohort and healthy controls (21, 22). Although both the aforementioned study (21, 22) and the current study were conducted over the course of several years (eliminating a seasonal effect), and we would not expect a difference based on center location (33), it is possible that systematic environmental and treatment differences (e.g., lighting, noise, depth of sedation) or differences in measurement techniques existed between the two cohorts.

In addition to the small sample size and heterogeneity within diagnostic subgroups and low numbers for longitudinal study, our study had other limitations. First, normal diurnal lighting patterns within the patient rooms were not strictly enforced. Nonetheless, we demonstrated that the maintenance of normal diurnal lighting patterns did not impact melatonin periodicity. This is congruent with findings in critically ill adults, who demonstrated lack of pineal responsiveness to light (39), and critically ill children who demonstrated loss of nocturnal serum melatonin peaks irrespective of lighting (23). Second, we were unable to control many PICU exposures, including opioids, benzodiazepines, and vasoactive agents given their

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near-ubiquitous use in critical care, which may all impact melatonin serum levels (11, 14). Finally, enrollment was slow and, although we do not believe that slow enrollment affected the validity of the results herein, the enrollment limitations would hinder the feasibility of a larger study, particularly if single center. It is worth noting that this is the largest single cohort examining melatonin in critically ill children.

CONCLUSIONS

Periodicity of and total daily aMT6s excretion is heterogeneously maintained early in pediatric critical illness. However, this may not hold true for specific diagnostic categories. Further longitudinal studies involving strict controls to isolate the influence of selected disease-related, therapeutic, and environmental factors are warranted.

This work related to patients was performed at Children's Hospital, London Health Sciences Centre in London, ON, Canada. Sample storage and analysis was performed at Translational Research Institute, London, ON, Canada.

Supported, in part, from the Children's Health Research Institute Internal Research Fund Grant ($7000) and Lawson Health Research Institute Internal Research Fund grant ($14,000).

Dr. Foster received funding from Children's Health Research Institute and Lawson Health Research Institute. The remaining authors have disclosed that they do not have any potential conflicts of interest.

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