Disturbed Sleep in Pediatric Patients With Leukemia: The Potential Role of *Interleukin-6* (–174GC) and *Tumor Necrosis Factor* (–308GA) Polymorphism

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Purpose/Objectives: To explore an association between sleep quality in children and adolescents undergoing therapy for acute lymphoblastic leukemia (ALL) and polymorphisms in two proinflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor (TNF).

Design: Retrospective exploratory analysis using data from a multi-institutional prospective study comparing objective sleep measures by actigraphy over 10 days with retrospective genotyping of *IL-6* (–174GC) and *TNF* (–308GA).

Setting: Pediatric oncology centers in the southeastern and southwestern United States and in Canada.

Sample: 88 children or adolescents with ALL.

Methods: Secondary analysis of 88 patients (ages 5–18) with sleep quality measured by actigraphy over 10 days in their home environment and retrospective DNA genotyping.

Main Research Variables: Sleep variables and genotype.

Findings: *IL-6* promoter (–174G>C) C allele was associated with fewer total daily sleep minutes ($p = 0.028$) and fewer daily nap minutes ($p < 0.01$). Patients with the *TNF* genotype AA had 28.2 more minutes of wake after sleep onset ($p = 0.015$), 3.4 more nocturnal wake episodes ($p = 0.026$), and a 5% lower sleep efficiency rate ($p = 0.03$) than their GA genotype counterparts.

Conclusions: Patients with the *TNF* (–308G>A) or *IL-6* (–174G>C) polymorphisms demonstrated disturbed sleep. This study is the first to find a relationship between these two cytokines and disturbed sleep in children and adolescents with cancer.

Implications for Nursing: Disturbed sleep among pediatric patients with cancer is multifactoral and includes interactions among environment, medications, and genotype. Additional research should explore serum proinflammatory cytokine levels and the influence of mood and worry on sleep.

Pediatric patients with cancer have rated disrupted sleep and fatigue as two of the most distressing symptoms related to their illness and treatment (Hinds et al., 1999; Hinds, Hockenberry, Gattuso, et al., 2007). These disturbances can persist for years after treatment for acute lymphoblastic leukemia (ALL), with as many as 50% of ALL survivors reporting sleep problems more than 10 years after completion of anticancer therapy (Meeske, Siegel, Globe, Mack, & Bernstein, 2005). Sleep disturbances in patients with cancer are common but often are undiagnosed or assumed to be a tolerable side effect of cancer or its treatment (Rosen, Shor, & Geller, 2008). Several studies have demonstrated poor sleep efficiency in pediatric patients undergoing cancer treatment, with an average sleep efficiency of 84% in patients with ALL who were in their home environment and 72% in patients with acute myelogenous leukemia or solid tumors who were in a hospital environment (Hinds, Hockenberry, Gattuso, et al., 2007; Hinds, Hockenberry, Rai, Zhang, Razzouk, Cremer, et al., 2007; Hinds, Hockenberry, Rai, Zhang, Razzouk, McCarthy, et al., 2007). Hinds, Hockenberry, Gattuso, et al. (2007) documented poor sleep quality and high levels of fatigue in children and adolescents at home during continuation chemotherapy for ALL. The study found that these patients stayed in bed longer, had poorer sleep efficiency, and had more nocturnal awakenings than cohorts of healthy children and adolescents. Given the detrimental effects of poor sleep on the immune system and cognitive development, the authors of this current article believe that decreased sleep quality is a crucial area to explore in pediatric patients with cancer. Therefore, the authors proposed a biobehavioral model of disrupted sleep in that genetic variability of selected inflammatory mediators might predict at-risk pediatric patients with cancer. The study framework was the Human Response Model, which integrates biopsychological factors, individual characteristics that may or may

not be modifiable, environmental factors that represent potential risks or factors of influence on adaptation, and individual adaptations or human responses to altered health states and to therapeutic interventions designed to improve health states (Heitkemper, Levy, Jarrett, & Bond, 1995; Heitkemper & Shaver, 1989).

Biobehavioral Mechanisms Related to Disrupted Sleep

Clinical researchers have proposed that a shared biological mechanism, such as a cytokine-neuroimmunologic model, may explain certain debilitating symptoms of cancer and cancer-related treatment (Cleeland et al., 2003; Lee et al., 2004). Animal models and clinical evidence suggest that fatigue and sleep impairment may be mediated by cytokines acting on the peripheral and central nervous systems (Cleeland et al., 2003). Two proinflammatory cytokines, interleukin-6 (IL-6) and tumor necrosis factor (TNF), regulate acute-phase immune reactions and interactions and have been correlated with these cancer-related symptoms (Kurzrock, 1997; Wood, Nail, Gilster, Winters, & Elsea, 2006). IL-6 has been noted to cause "sickness syndrome" in animal models, including disrupted sleep, decreased voluntary wheel running, and anorexia (Wood et al., 2006), and has been directly correlated with fever, weight loss, and night sweats in patients with lymphoma (Kurzrock, 1997). Together, high serum concentrations of IL-6 and TNF have been implicated in cancer-related depression, anemia, weight loss, and cachexia and may indicate poorer prognosis and outcome (Kurzrock, 1997; Lee et al., 2004; Wood et al., 2006).

IL-6 and TNF also have been correlated with debilitating fatigue and sleep disturbances in patients with chronic illnesses other than cancer, including obstructive sleep apnea, narcolepsy, insomnia, multiple sclerosis, HIV, chronic inflammatory diseases, systemic arthritis, and obesity (Belluco et al., 2003; Fayad, Cabanillas, Talpaz, McLaughlin, & Kurzrock, 1998; Hoffmann et al., 2001; Kurzrock, 1997; Rich et al., 2005; Vgontzas et al., 2005). Studies by Vgontzas et al. (1999, 2005) have shown that serum IL-6 mediates excessive sleepiness, and the morning serum level of IL-6 has been negatively correlated with the depth and quantity of sleep from the previous night in adults. Sleep deprivation of two hours per night for one week led to increased serum levels of IL-6 and TNF in healthy volunteers (Vgontzas, Zoumakis, Bixler, et al., 2004). In addition, morning levels of serum IL-6 were positively correlated with rapid eye movement sleep latency and percentage of wake after sleep onset (WASO) and negatively related to sleep efficiency after controlling for race, age, gender, and body mass index in healthy adults (Hong, Mills, Loredo, Adler, & Dimsdale, 2005). The dose-limiting toxicity of exogenous administration of TNF is extreme fatigue, and administration of a TNF antagonist causes a marked decrease in sleepiness (Schiller et al., 1991; Vgontzas, Zoumakis, Lin, et al., 2004). These findings suggest that sleep deprivation results in an increased inflammatory response and subsequent symptoms of sleep disturbance, fatigue, and cognitive impairment are most likely modulated by IL-6 and TNF.

Genetic Predispositions

Interleukin-6 and Tumor Necrosis Factor Levels

An individual's genetic predisposition may influence IL-6 and TNF serum levels. The *IL-6* gene, located in the short arm of chromosome 7 (7p21), displays a single nucleotide polymorphism (SNP) in the promoter region (–174GC) with the common allele (wild-type allele) guanine (G) and the minor allele cytosine (C). An association with the *IL-6* gene expression has been found to correlate with serum levels (Fishman et al., 1998). In a study of healthy adults, those volunteers with the *IL-6* (–174GC) genotype of GG or GC had leukocytes that produced IL-6 levels that were three times higher than those of volunteers with the genotype of CC (Hoffmann et al., 2001). In adults with colorectal cancer, the absence of the *IL-6* gene promoter (–174G>C) polymorphism correlated with higher IL-6 serum levels (Belluco et al., 2003). The *TNF* gene, located in the short arm of chromosome 6 (p21.3), displays an SNP in the promoter region (–308G>A) with the common allele (wild-type allele) G and the minor allele adenine (A). The TNF SNP has been correlated with TNF levels, with the homozygous AA genotype associated with higher TNF production than the GG genotype (Wilson, Symons, McDowell, McDevitt, & Duff, 1997). In addition, the prevalence of the –308A allele is higher in patients evaluated for obstructive sleep apnea than in the age-matched population (Riha et al., 2005). Because of the systemic effects of increased IL-6 and TNF serum levels, these polymorphisms are likely part of the mechanism of sleep disturbances in patients with cancer.

The purpose of this study was to determine whether an association exists between sleep quality and *IL-6* or *TNF* polymorphisms in children and adolescents undergoing therapy for ALL. To the authors' knowledge, this study is the first exploring the relationship between cytokine polymorphisms and sleep disturbance in pediatric patients with cancer.

Methods

Patients

Patients were recruited from a previously described multi-institutional, prospective study of sleep and fatigue

in children and adolescents with low- or standard-risk ALL during continuation chemotherapy (Hinds, Hockenberry, Gattuso, et al., 2007). Of the 100 patients enrolled in the study, 12 had unavailable actigraph data caused by actigraph failure or insufficient recording. This loss of data is less than the typical failure rate reported in the pediatric literature (Glaze, 2004). Therefore, 88 patients are included in this association study of genotype and sleep variables. The study was approved by the institutional review board of each participating institution, and informed consent was obtained.

Evaluation of Patient Sleep Quality

Patients wore an actigraph during a 10-day period, and the resulting data were analyzed via software using Sadeh's algorithm (Sadeh, Sharkey, & Carskadon, 1994). During the 10-day period, patients spent every night in their home sleep environment and they received dexamethasone, according to their ALL protocol, on days 6–10 for an off/on dexamethasone comparison. Patients continued with weekly complete blood count and chemotherapy per protocol.

Genotyping for the *Interleukin-6* and *Tumor Necrosis Factor* Polymorphisms

DNA that was previously extracted by standard techniques from peripheral blood leucocytes was analyzed for *IL-6* and *TNF* polymorphisms. Polymerase chain reaction (PCR)-restriction fragment length polymorphism assays of the 164bp *IL-6* promoter and the 257bp *TNF*-α promoter were performed for genotyping. *IL-6* (–174GC) was amplified by using the fluorescently labeled primer 5′–FAM–GCC TCA ATG ACG ACC TAA GC–3′ with 5′–TCA TGG GAA AAT CCC ACA TT–3′. The PCR mixture comprised 50 mcM of each deoxyribonucleotide triphosphate, 1.5 mM MgCl2, 0.5 units Taq (a thermostable DNA polymerase), 1 mcM of each primer, 1 × reaction buffer, and 40 ng genomic DNA in total reaction volume of 20 mcl. PCR conditions were as follows: one cycle of five minutes at 95°C followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 58°C, and 40 seconds at 72°C; finally, one cycle of 10 minutes at 72°C. For *IL-6* (–174GC) genotyping, 2 units of DNA fragments were digested by NlaIII for 16 hours at 37°C. The amplified G allele variant was digested once, leaving one large fragment of 163bp, whereas the C allele was cleaved twice resulting in two fragments of 111bp and 52bp, respectively. For *TNF* –308GA genotyping, the PCR product was digested by 2 units of NcoI for 16 hours at 37°C. Only the PCR product with –308G allele can be cut into one large fragment of 237/241bp, whereas the –308A allele cannot be cleaved by NcoI. The digested DNA fragments were identified following capillary electrophoresis by ABI3730xl, analyzed by using GeneMapper® software, and then were confirmed visually.

Statistical Analyses

The sleep quality data analyses have been previously described (Hinds, Hockenberry, Gattuso, et al., 2007). Allele frequencies were calculated for the 88 patients and stratified according to race to determine genotype variability specific to race. Genotypes were pooled into binary groups for statistical analyses to test for dominant or recessive genetic effect. Therefore, the genotypes were grouped as homogenous common allele versus genotypes containing one minor allele and homogenous minor allele versus genotypes containing one common allele. The association between sleep variables and genotypes was analyzed. First, the association between sleep variables and each patient characteristic variables was tested in univariate models for longitudinal data. Next, the association between sleep variables and genotypes was studied through multiple regression modeling after controlling for the significant variables from the univariate models and the dexamethasone usage (calculated by using the Proc Mixed procedure [SAS®, version 9.1.3]). The week-specific compound symmetry dependence structure was chosen by using the Akaike information criterion to illustrate the intrapatient correlation (Littell, 2006). Patient characteristics considered included risk group (Children's Oncology Group [COG] low risk, COG standard risk, St. Jude low risk, St. Jude standard risk), age (continuous), gender, and race (Caucasian, African American, or other). Standard- and low-risk groups are based on disease

Table 1. Participant Characteristics

Characteristic	n	%
Gender		
Male	54	61
Female	34	39
Age at enrollment (years)		
Younger than 7	26	30
7–12	50	57
13 or older	12	14
Race		
Caucasian	72	82
African American	11	13
Asian	1	1
Other	4	5
Protocol, ALL risk group		
St. Jude, low	22	25
St. Jude, standard	27	31
COG, low	14	16
COG, standard	25	28
Enrollment site		
St. Jude Children's Research Hospital	49	56
Texas Children's Cancer Center	27	31
The Hospital for Sick Children	12	14

N = 88

ALL—acute lymphoblastic leukemia; COG—Children's Oncology Group

Note. Because of rounding, not all percentages total 100.

characteristics such as age at presentation, white blood cell count at presentation, and response rate to induction chemotherapy. The different treating institutions, COG and St. Jude, have slightly different dosing of steroids for their treatments groups. Risk group, therefore, is an indicator for steroid dosage. The interaction terms between genotypes and off/on dexamethasone also were considered. Residual analysis was carried out to confirm the appropriate model assumptions. The least squares mean (LSMEAN) for specific genotypes after adjusting for other explanatory variables were reported from the longitudinal models. The criterion for significance for all analyses was a p value below 0.05. All analyses were performed with SAS®, version 9.1.3.

Results

Demographic information is presented in Table 1. Allele frequencies of *IL-6* and *TNF* are depicted in Table 2. The authors found the frequency of the *IL-6* (–174G>C) G common (wild-type) allele frequency to be 0.68 within this patient group; however, when the patients were stratified by race, the G allele had a frequency of 0.63 among Caucasians and a frequency of 1 among African Americans. The frequency of the *TNF* G allele was approximately 0.68 across all racial groups. *TNF* promoter polymorphism (–308G>A) was in Hardy-Weinberg equilibrium, therefore limiting the possibility of genotype error. The authors found no gene-gene interactions (influence in expression) between *IL-6* and *TNF* in any analysis.

Table 2. Frequency of *Interleukin-6* and *Tumor Necrosis Factor* Genotype Based on Race in Pediatric Patients With Acute Lymphoblastic Leukemia

	Frequency of Genotype by Racial Group			
Variable	Total Patients (N = 88)	Caucasian (N = 72)	African American (N = 11)[a]	Other (N = 5)
Interleukin-6				
G (n = 120)	0.6818	0.6319	1	0.7
C (n = 56)	0.3182	0.3681	–	0.3
GG (n = 42)	0.4773	0.3889	1	0.6
GC (n = 36)	0.4091	0.4861	–	0.2
CC (n = 10)	0.1136	0.125	–	0.2
Tumor necrosis factor				
G (n = 120)	0.6818	0.6806	0.6818	0.7
A (n = 56)	0.3182	0.3194	0.3182	0.3
GG (n = 48)	0.5455	0.5417	0.5455	0.6
GA (n = 24)	0.2727	0.2778	0.2727	0.2
AA (n = 16)	0.1818	0.1806	0.1818	0.2

[a] African Americans, when genotyped, only had the *interleukin-6* G allele, therefore, that participant population all had the GG genotype. African Americans in this study had the same frequency for the *tumor necrosis factor* allele and genotype as Caucasians and other populations.

A—adenine; C—cytosine; G—guanine

Note. A total of 176 alleles (G, C, and A) were reported from 88 participants. Each participant had one genotype (GG, GC, CC, GA, or AA).

Overall association tests between sleep and genotype are presented in Table 3. In the 10 days of actigraphy monitoring and controlling for age, risk group, and off/on dexamethasone, the *IL-6* promoter (–174G>C) C allele was associated with about 27 fewer total daily sleep minutes ($p = 0.028$) and about 15 fewer daily nap minutes ($p < 0.01$). The *TNF* (–308G>A) gene was significantly associated with WASO ($p = 0.049$). The *TNF* G allele was associated with significantly more nocturnal awakenings during the on-dexamethasone week than during the off-dexamethasone week ($p = 0.023$).

Table 4 illustrates the LSMEAN values of the actigraph measurements after adjustment for the possible related confounders of age, gender, risk group, and chemotherapy week. Patients with the *TNF* genotype AA had a WASO LSMEAN of 94 minutes, and those with the GA genotype had a WASO LSMEAN of 66 minutes. This difference of 28 minutes is significantly different from zero ($p = 0.015$). Patients with the AA genotype also had significantly more nocturnal awakenings (LSMEAN = 16.7) than those with the GA genotype (LSMEAN = 13.3, $p = 0.026$). In addition, after adjusting for other significant explanatory variables, patients with the AA genotype had a lower sleep efficiency estimate (82%) than their GA counterparts (88%, $p = 0.03$). To be sure that the sleep disturbances the authors observed were associated with the genotype, other factors were examined that could have confounded the analyses by affecting sleep and fatigue levels. The authors noted that 53 different medications were administered during the period of data collection, but they were routine cancer-directed and supportive medications. No pharmacologic sleep aids were used by these patients. In addition, hemoglobin values ranged from 9.1–13.8 g/dl, and no patient received a transfusion. Similarly, albumin was measured on the first day of dexamethasone, and ranges also were normal (median = 4.3 g/dl; range 3–5.5 g/dl). No concurrent illnesses were observed, and all patients were treated as outpatients throughout data collection—sleeping at their own homes or medical domiciles as they were accustomed to doing.

Discussion

In cohorts of healthy Caucasian adults, the frequency of the *IL-6* (–174G>C) polymorphism was found to be 0.57–0.68, which is comparable to the authors' frequency of 0.68 (Cox et al., 2001; Fishman et al., 1998; Hegedus, Skibola, Bracci, Holly, & Smith, 2007). The *IL-6* polymorphism varies across races; the frequency of the G allele in African American and African-Caribbean populations (> 0.9) is higher than that in Caucasian populations (0.62)

Table 3. Association Tests Between Sleep Measures and *Interleukin-6* or *Tumor Necrosis Factor* Genotypes

Genotype	WASO	Total Daily Nap Time	Sleep Efficiency	Actual Sleep Time	Total Daily Sleep	Sleep Duration	Nocturnal Awakenings
Interleukin-6	0.1163	0.0292*	0.2129	0.1838	0.071	0.4232	0.822
Interleukin-6 G+	0.1125	0.1567	0.1368	0.3165	0.2093	0.5606	0.5315
Interleukin-6 C+	0.0781	0.0094*	0.1747	0.0785	0.0278*	0.197	0.848
Tumor necrosis factor	0.0493*	0.4204	0.0882	0.3348	0.3883	0.4417	0.0644
Tumor necrosis factor G+	0.0654	0.2206	0.1009	0.1395	0.9536	0.5562	0.0233*
Tumor necrosis factor A+	0.725	0.8161	0.7289	0.3983	0.2618	0.2034	0.48

* $p < 0.05$

A—adenine; C—cytosine; G—guanine; WASO—wake after sleep onset

(Hoffmann et al., 2002). In the current group, none of the 11 African American patients carried a C allele. The *TNF* (–308G>A) G allele has a frequency of 0.84–0.9 in healthy adults, but it was 0.68 in the current study. No differences in *TNF* polymorphism across racial groups have been described in the literature (Hoffmann et al., 2002; Jeanmonod, von Kanel, Maly, & Fischer, 2004). The *TNF* (–308G>A) A allele is more prevalent in the authors' pediatric ALL population than in healthy adult populations (0.32 versus 0.1–0.16). Associations have been reported between the *TNF* (–308G>A) A allele in adults and increased risk of hematologic cancers such as lymphoma and leukemia (Au, Fung, Wong, Chan, & Liang, 2006; Bel Hadj Jrad et al., 2007; Cerhan et al., 2008; Duarte et al., 2005; Guo, Wang, Li, & Zhang, 2005; Wang et al., 2006). This may explain the different A allele frequency and Hardy-Weinberg equilibrium in the authors' select pediatric leukemia population.

Pediatric patients in the current study with the *IL-6* (–174G>C) C allele had fewer total sleep minutes and less nap time (after controlling for age, risk group, gender, and off/on dexamethasone status) than did the patients who did not express the C allele. Fewer total sleep minutes and less nap time were findings reported in the parent study and were associated with restorative sleep and less time in bed (Hinds, Hockenberry, Gattuso, et al., 2007). One explanation for this decreased sleep and nap time is that patients with C allele express less serum IL-6, a known mediator of sleepiness and a key player in the homeostatic drive for sleep (Vgontzas et al., 2005). Therefore, patients who express lower levels of IL-6 may feel less need to nap and have slightly decreased sleep duration needs. Under the same rationale, patients with higher levels of IL-6 may have felt more tired and driven to sleep, therefore falling asleep faster at bedtime. This would result in higher sleep efficiency, as the proportion of time spent in bed actually sleeping is greater, and patients would have a stronger homeostatic drive for continual sleep, therefore experiencing fewer nocturnal awakenings. This explanation is based on the theory that the *IL-6* (–174G>C) G allele produces higher in vivo and in vitro cytokine levels than other possible alleles at these positions (Belluco et al., 2003; Hoffmann et al., 2001).

Evidence in the literature also suggests that *TNF* (–308G>A) minor A allele produces higher in vitro cytokine levels (Wilson et al., 1997). The patients in the current study with the *TNF* promoter AA genotype had lower sleep efficiency, longer WASO, and more nocturnal awakenings than their GA or GG counterparts. These findings may provide insight into the mechanism of increased fatigue found in patients with cancer who express the *TNF* (–308G>A) polymorphism or have elevated serum levels of TNF (Bower, Ganz, Aziz, & Fahey, 2002; Kurzrock, 2001; Schubert, Hong, Natarajan, Mills, & Dimsdale, 2007). The authors hypothesize that patients with increased production of TNF (i.e., those with the homozygous AA genotype) have increased inflammation leading to poorer sleep efficiency, more sleep disturbance, and, ultimately, increased feelings of fatigue. Lastly, the administration of dexamethasone and its known disruption on sleep and fatigue appears to play a role in the difference found in the number of nocturnal awakenings for those with the *TNF* G allele, having significantly more nocturnal awakenings during the on-dexamethasone week than during the off-dexamethasone week ($p = 0.023$).

To the authors' knowledge, only one study has examined sleep disturbances and *TNF* (–308G>A) in adult patients with cancer and their caretakers (Aouizerat et al., 2009). Aouizerat et al. (2009) found that study participants with either the AA or AG genotype had a lower Generalized Sleep Disturbance Scale (GSDS) score than their GG counterparts. That study is difficult to compare because the focus of the current one is on pediatric patients with cancer with objective sleep data. The analyses by Aouizerat et al. (2009) included adult patients (who had a variety of cancers and treatments) and their healthy family caretakers, and sleep quality was measured by using the GSDS questionnaire. In addition, the grouping of analyses in Aouizerat et al. (2009) was by AA plus AG genotype participants versus GG genotype participants because of the low frequency of the A allele in that cohort.

Table 4. Least Squares Mean Values of Actigraph Measurements After Adjustment for the Possible Related Confounders of Age, Gender, Risk Group, and Off or On Dexamethasone

Variable and Genotype	Estimate	SE	Difference	SE	p[a]
TNF (–308G>A)					
Wake after sleep onset					
AA	93.9	8.6	Reference	Reference	–
GA	65.6	7.3	–28.3	11.4	0.0155
GG	79.9	5.1	–13.9	9.9	0.1645
Nocturnal awakenings					
AA	16.7	1.1	Reference	Reference	–
GA	13.3	1	3.4	1.5	0.0264
GG	13.9	0.7	2.7	1.3	0.0408
Sleep efficiency					
AA	82.1	1.8	Reference	Reference	–
GA	87.5	1.6	5.4	2.4	0.03
GG	84.7	1.1	2.6	2.1	0.2218
Variable and Genotype	**Estimate**	**SE**	**Difference**	**SE**	**p[a]**
IL-6 (–174G>C)					
Total nap minutes					
GG	46.1	4.2	Reference	Reference	–
GC	32.2	4.4	13.9	6.1	0.0242
CC	26.7	8.6	19.4	9.4	0.0425
C+	31	3.9	15.1	5.7	0.0094
Total daily sleep minutes					
GG	95.2	10.5	Reference	Reference	–
GC	571.3	12.9	24.3	12.4	0.0533
CC	559.1	18.5	36.5	18.8	0.055
C+	568.5	12.2	26.6	11.9	0.0278

[a] p values for each genotype estimate compared to reference genotype. *IL-6* GG and *TNF* AA are considered the higher expressing genotypes.

A—adenine; C—cytosine; G—guanine; IL-6—interleukin-6; SE—standard error; TNF—tumor necrosis factor

Note. Estimates adjusted for possible confounders include wake after sleep onset, gender, and risk; nocturnal awakenings, age, gender, risk, and chemotherapy week; efficiency, risk, and gender; total nap minutes and gender; total daily sleep minutes, age, gender, and risk; and sleep minutes, age, gender, and risk.

Limitations

The retrospective exploratory analysis presented here has some limitations, such as a small sample size, and larger numbers will need to be assessed to account for gene-environment interactions. One of the most significant limitations of the current study is that it is unclear whether the genotype can accurately be used as surrogates for the expected serum cytokine levels of Il-6 or TNF. To address this, the authors attempted to retrospectively measure serum cytokine levels to confirm that these polymorphisms are reflective of actual cytokine levels; however, the serum cytokine levels in the stored samples were primarily undetectable, most likely from degradation caused by processing, shipping, and storage—all common obstacles. Although the detection of cytokine polymorphisms in serum and genetic material is a rapidly expanding area of research, interpreting study findings is still difficult: conflicting results; small sample sizes; difficulty obtaining exact serum measurements because of short half lives and diurnal variations in cytokine release; and the use of different assays, dilutions, and processing methods all have contributed to an inability to compare findings among studies.

Future research should not only explore the associated serum cytokine levels, but should explore other polymorphisms, more specifically those within the *TNF* locus that are known to affect cytokine secretion. In addition, the study warrants replication within larger samples with ALL, and the exploration of other variables such as the impact of mood and worry on sleep.

Nursing Implications

This study suggests that a biological mechanism may contribute to disrupted sleep in pediatric patients with ALL. The authors found that patients with genotypes associated with higher gene expression of IL-6 and TNF have increased sleep difficulty and longer time in bed. With this knowledge, nurses should assess sleep history including time to bed, awakening, and daytime sleepiness. Nurses should emphasize the importance of good sleep hygiene, including the limitation of activity prior to bedtime and the establishment of a habitual bedtime and morning awakening. This assessment is most important for the adolescents who are known within the general population to have a higher prevalence of delayed sleep phase-type disorder (Pelayo, Thorpy, & Glovinsky, 1988). In addition, sleep deprivation is common during therapy and hospitalization, resulting in increased inflammatory response, symptoms of sleep disturbance, and fatigue and cognitive impairment. These symptoms may be further influenced by genotypes of proinflammatory cytokines, as described. The biobehavioral model provides an appropriate framework for the development of future exploration of additional genotypes and levels of proinflammatory cytokines that may contribute to increased sleep, fatigue, and daytime function.

Conclusion

The authors have found that children with ALL and the *IL-6* (–174G>C) G common allele have greater sleep

needs and time in bed, whereas the *IL-6* (–174G>C) C allele is associated with decreased sleep drive with less total sleep and nap minutes. *TNF* (–308 G>A) A minor allele was found to contribute to disturbed sleep by increasing WASO and nocturnal awakenings and lowering sleep efficiency. As the importance of sleep disruption and its detrimental effects on health, including immune function, are brought to light, understanding the roles of these two proinflammatory cytokines in cancer treatment and related disturbances in sleep will be crucial.

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Digital Object Identifier: 10.1188/11.ONF.E365-E372

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