Biological Research For Nursing

Influence of Inflammatory and Oxidative Stress Pathways on Longitudinal Symptom Experiences in Children with Leukemia

Journal:	*Biological Research for Nursing*
Manuscript ID	BRN-ROR-19-01-0010.R1
Manuscript Type:	Report of Original Research
Keywords:	childhood leukemia, symptom trajectory, oxidative stress biomarker, inflammatory biomarker, longitudinal parallel process

Abstract

Purpose: The purpose of this study was to explore the influence of oxidative stress (F2-isoprostanes) and inflammatory (IL-8) biomarkers on symptom trajectories during the first 18 months of childhood leukemia treatment.

Method: A repeated-measures design was used to evaluate symptom experienced by 218 children during treatment. A symptom cluster (fatigue, pain, and nausea) was explored over four time periods: initiation of post-induction therapy, 4 and 8 months post-induction therapy, and the beginning of maintenance therapy (12 months post induction). F2-isoprostanes and IL-8 were evaluated in cerebrospinal fluid (CSF) samples collected at ~~the~~ baseline (diagnosis) and then at the subsequent four time periods. The longitudinal relationships of th~~e~~ese biomarkers with the symptom cluster were examined using the longitudinal parallel process.

Results: Pain and fatigue levels were highest during the post-induction phases of treatment, and then decreased slightly during maintenance therapy; while nausea scores were relatively stable. Even in the later phases of treatment, children continued to experience symptoms. CSF levels of the biomarkers increased during the post-induction phases of treatment. Early increases in the biomarkers were associated with more severe symptom during the same period; patients who had increased biomarkers over time also experienced more severe symptoms over time.

Conclusions: Findings reveal that children experience symptoms throughout the course of leukemia treatment, and support hypothesized longitudinal relationships of oxidative stress and inflammatory biomarkers with symptom severity. Activation of the biomarker pathways during treatment may explain underlying mechanisms of symptom experiences and identify which children are at risk for severe symptoms.

Keywords: childhood leukemia; symptom trajectory; oxidative stress biomarker; inflammatory biomarker; longitudinal parallel process

Introduction

Childhood leukemia treatment over the last three decades has resulted in survival rates that exceed 80% (Hunger et al., 2012; Tasian et al., 2015). Intensity of treatment required for cure has created numerous side-effects that often are overlooked. Fatigue, pain, nausea, depression, sleep disturbances, mental exhaustion and changes in activity are the most common treatment-related symptoms that can profoundly impact children's lives. Up to 80% of children with cancer experience at least one symptom during treatment but on average children experience more than 10 symptoms during therapy (Baggott et al., 2012~~; Yeh et al., 2008~~). Treatment-related pain, nausea, and fatigue (Hedstrom et al., 2003) were the most common symptoms in a group of 121 children with cancer. Physical symptoms were common (prevalence > 35%) in a group of 160 children with cancer and included lack of energy, pain, drowsiness, nausea, cough, and lack of appetite (Collins et al., 2000). Parents of cancer patients and health care professionals reported by proxy that cancer-related fatigue was a frequent symptom experienced during treatment (Gibson et al., 2005). In a study of health-related quality of life (HRQOL) in 61 children following myelosuppressive chemotherapy, those with poorer functional status and higher symptom burden were associated with significant decreases in HRQOL; children experienced on average, 10.6 separate symptoms. The most common symptoms included lack of energy, pain, feeling drowsy, nausea, and feeling sad and irritable (Baggott et al., 2010). In a recent published study by this research team, children with leukemia 3 to 18 years old experienced sleep disturbance and nausea that had limited change over time. Fatigue, pain, and depression decreased over four time periods; however, none of the symptoms completely resolved over time (Hockenberry et al., 2017).

The majority of childhood cancer symptom research has been among heterogeneous groupings of pediatric oncology diagnoses (Hinds et al., 2007), which limits ability to tailor inventions for children, as specific diagnoses and treatment modalities are likely associated with

differing symptom profiles. There is limited understanding of the underlying mechanisms associated with symptom severity and why significant variations occur even when disease and treatment are similar. Based on our prior work, we focused on the selected~~The potential influence of~~ inflammatory and oxidative stress responses during the most intensive phase of childhood leukemia treatment that may influence symptom toxicities and should be explored (Caron et al., 2009; Hockenberry et al., 2014, 2015; Moore et al., 2015, 2018; Rodgers et al., 2016; Stenzel et al., 2010).

I~~The i~~nflammatory pathways are ~~is~~ designed to clear an organism of the cause of cell injury (e.g. microorganisms, toxins) and the consequences of injury (e.g. necrotic cells, tissues). Inflammation is controlled by a host of extracellular mediators and regulators, including cytokines which act as key modulators of inflammation (Turner et al., 2014). IL-8 (CXCL8) is a critical inflammatory mediator and is elevated in many chronic inflammatory conditions found in adults; important examples include psoriasis, adult respiratory syndrome, chronic obstructive pulmonary disease, diabetes, chronic liver disease, polyarthritis, and asthma. Investigators report that in various cancers upregulation of IL-8 is often correlated with disease activity (Donnelly et al., 1993; Koch et al., 1991; Nakamura et al., 1992; Qiao et al., 2016; Zimmermann et al., 2011). Increased inflammatory biomarkers have also been observed in children with numerous illnesses such as juvenile idiopathic arthritis (Sznurkowska et al., 2018), acute kidney injury (Greenberg et al., 2018), and children who are maltreated (Coelho et al., 2014). IL-8 shows recent promise as a biomarker for acute child and adolescent psychopathology (Gariup et al., 2015).

Oxidative stress refers to cellular injury and degeneration caused by reactive oxygen species (ROS). ROS are reactive because they act as an electron acceptor and “steal” electrons from other molecules. These compounds damage cell structures, especially lipids. Compared to other organs, the brain is particularly vulnerable to oxidative stress due to limited antioxidant capacity, greater energy requirements, and higher lipid content (Floyd, 1999).

Methotrexate, a drug given by multiple routes (intravenous, oral, and intrathecal) and commonly used for acute lymphoblastic leukemia (ALL) treatment, decreases antioxidant defense resulting in increased oxidative stress, and methotrexate-induced oxidative stress has been observed in the brain (Hockenberry et al., 2014). ROS have been linked to the pathophysiology of numerous disorders including traumatic brain injury, ischemia-reperfusion injury, atherosclerosis, inflammatory diseases, cancer, adriamycin-induced cardiomyopathy, neuro-degenerative conditions, and age-related functional decline (Hannun, 1996; Liou et al., 2003; Margaill et al., 2005; Minuz et al., 2006; Montine et al., 2005; Pellegrini-Giampietro et al., 1990; Pratico et al., 1998; Pyne-Geithman et al., 2005; Shohami et al., 1997; Weber, 1994). Two published studies evaluated an oxidative stress measure, total antioxidant capacity (TAC), in children with cancer. TAC was evaluated in 20 children with cancer at the time of diagnosis and during the first three cycles of chemotherapy and revealed significant decreases in TAC over time, suggesting an oxidative stress additive effect that could impact response to treatment, disease course, and prognosis (Papageorgiou et al., 2005). In another study of 13 children with leukemia and various solid tumors, TAC was impaired in both groups; however, higher oxidative stress occurred in the leukemia group (Mazor et al., 2008).

The purpose of this study was to explore the influence of inflammatory and oxidative stress biomarkers on specific symptom trajectories during the first 18 months of childhood leukemia treatment. Inflammatory and oxidative stress biomarkers were measured in cerebrospinal fluid (CSF) samples during diagnostic and therapeutic lumbar punctures at baseline. We hypothesized that increased levels of inflammatory and oxidative stress biomarkers would be associated with more severe symptom experiences during ALL treatment.

Conceptual Model

The conceptual framework for this study (Figure 1) proposed that the physiologic responses to leukemia treatment involve activation of inflammatory and oxidative stress pathways (measured by CSF biomarkers), which will influence the number and severity of

symptoms during childhood cancer therapy. Chemotherapy agents commonly used for childhood leukemia (e.g., methotrexate) can increase reactive oxygen species (ROS) production as a result of altered folate metabolism and decreased antioxidant enzyme production. These chemotherapy agents also are associated with an inflammatory response that induces cytokine release. A key factor underlying the organizational framework for this study is that individual phenotypic susceptibility to oxidative stress release and inflammatory response can contribute to subsequent clinical outcomes and symptom severity.

Methods

Design

A repeated-measures longitudinal design was used to evaluate treatment-related symptom associations experienced by 218 children and adolescents 3 to 18 years of age receiving ALL treatment. Symptom trajectories were explored over four time periods: initiation of post-induction therapy, 4 and 8 months during post-induction therapy, and the beginning of maintenance/continuation therapy (12 months post induction). Symptom measures included fatigue, pain, and nausea. Oxidative stress (F2-isoprostanes) and inflammatory (IL-8) biomarkers were measured in cerebrospinal fluid (CSF) samples obtained at diagnosis (baseline) and at four time points during ALL treatment (Times 1 through 4).

Settings and Sample

The settings for this study were four major childhood cancer treatment centers in the United States. When combined, the four centers diagnose more than 200 children and adolescents with leukemia who meet the study eligibility criteria each year. Children with a cognitive disability identified pre-leukemia diagnosis were ineligible for study enrollment.

The sample size for this study was 218. All the participants provided complete information about sociodemographic variables (age, sex, race/ethnicity) and leukemia risk category. Leukemia r~~R~~Risk category is determined by specific factors (biological and genetic

features of the leukemia cells, age, gender, and initial response to therapy) known to predict survival or relapse. There were intermittent, longitudinal missing data (3.7% to 28.4%) across all the time points among the longitudinal variables. Fortunately, Little's (1988) missing completely at random (MCAR) test showed that the missing data were MCAR ($\chi^2(1236) = 1223.82$, $p = .592$) and did not have a negative impact on parameter estimation (Little & Rubin, 2002). Thus, an expectation–maximization (EM) algorithm was performed for missing data imputation on the two baseline biomarker measures. Baseline biomarker measurements, sociodemographic variables, and leukemia risk category were controlled for as covariates in the main analysis. As for the longitudinal missing data on the biomarkers and the symptom measures from Time 1 to Time 4, they were automatically handled in multilevel modeling within the two-step longitudinal parallel-process technique described below.

Leukemia Treatment

Children were treated on an ALL protocol. Induction therapy (1 month) included weekly treatment with vincristine and daunomycin (for high-risk ALL), a corticosteroid and a dose of Peg-asparaginase, and two intrathecal methotrexate (IT MTX) treatments (Days 1 and 29). Post-induction therapy (6–8 months) involved several courses of treatment that included asparaginase, high- or intermediate-dose intravenous (IV) MTX (depending on ALL protocol assignment), vincristine, doxorubicin, corticosteroid, cytarabine, and mercaptopurine. During post-induction IT MTX was given on Day 1 of each 12-week cycle. Throughout therapy, study participants received CNS prophylaxis with standardized doses of IT MTX based on age. Maintenance/continuation therapy with the final study measurement, began 6 to 8 months after the end of induction therapy. Time intervals for each phase of treatment were influenced by participants' side effects and recovery from low blood counts.

Data Collection

Consent was obtained from a parent or legal guardian. Children (age seven years of age and older) and adolescents provided assent. Symptom assessments were obtained using

standardized instruments and occurred during a routine follow-up clinic visit; time points were chosen to evaluate the most intensive phase of therapy post-induction. CSF samples were collected in conjunction with the lumbar puncture for IT MTX treatment that coincided most closely with symptom data collection.

Measures

Symptom Measures and Cluster. Based on our previous findings (Hockenberry et al., 2017) we focused on the ~~3~~ three symptoms of fatigue, pain, and nausea. Symptoms were assessed using valid and reliable measures; data on each symptom was collected with a table PC (iPad). ~~This study used a~~ technological ~~n innovative approach to symptom assessment by measuring each symptom using a separate reliable and validated instrument (see Table 1) with a tablet PC (iPad).~~ English and Spanish versions of the instruments were available on the tablet PC. Standardized measures evaluated fatigue, pain, and nausea. For consistency across symptoms, parent proxy was used for the 3-6-year-old age group and symptom self-report for children ≥ 7 years of age. Specifically, fatigue was measured by three instruments ~~according~~ toused for ~~the~~ different age ranges: the Child Fatigue Scale (CFS; Hinds et al., 2010) for ages 7-12, the Adolescent Fatigue Scale (FSA; Mandrell et al., 2011) for ages 13-18, and the Parent Fatigue Scale (PFS; Hockenberry et al., 2003) fro ages 3-6. Then, all the scores from the three instruments were converted to comparable, standardized T-scores. All symptoms were reported over the past 2-4 weeks. Data collection required less than 25 minutes to complete at each time point. For each symptom scale, a higher score indicates greater symptom severity. Scores were used to determine symptom severity and classified into different symptom profiles labeled as mild, moderate and severe ~~(see Table 1)~~.

Because the three symptom measures (fatigue, pain, and nausea) were highly correlated with each other at each time point (Hockenberry et al., 2017), they were combined as a symptom cluster~~.~~ through exploratory factor analysis with maximum likelihood estimation

which returned a one-factor solution with significant factor loadings from .35 to .88 and at least 54% of variance explained at each time point (Table 14).

CSF cytokine measures. Two milliliters of CSF were obtained in conjunction with diagnostic and therapeutic lumbar punctures as part of clinical treatment. CSF samples were placed on ice and processed within 2 hours of collection to prevent reactions such as auto-oxidation. Samples were centrifuged at 4°C to remove any particulates, aliquoted and frozen at -80°C, and shipped on dry ice to the core research lab for this study. IL-8 was measured using a cytokine-specific, AlphaLISA immunoassay kit (Perkin-Elmer) as instructed by the manufacturer. This is a luminescent, homogenous (no wash) proximity-based assay with extremely high sensitivity and a wide (4 logs) dynamic range. Each sample was assayed in triplicate wells of 96-well, half-area white plates. Briefly, each well contained 10 µL of blank (artificial CSF with Prionex), sample, or standard and 20 µL of Acceptor Bead Solution. Once the Acceptor Bead Solution was added to the plate, it was sealed and incubated at room temperature for 1-hour. After 1-hour and under low light, 20 µL of Donor Bead Solution was added to each well and incubated at room temperature for 30-minutes. The plate seal was removed under low light and plates were read at 615 nm wavelength using a Perkin-Elmer Envision multilabel plate reader equipped with the Alpha option. Cytokine concentrations were determined by comparison to a standard curve of recombinant cytokine, using a linear regression logistic equation, and expressed as pg/ml.

CSF oxidative stress measures. CSF samples for the oxidative stress biomarker were obtained at the same time as the cytokine samples, processed, and stored as described above. F2-isoprostanes (F_2-IsoPs) are a sensitive non-invasive index of oxidative stress *in vivo*, and were measured by a competitive enzyme-linked immunoassay (ELISA) kit according to instructions (Cayman Chemical). The kit was designed for extraction-free analysis of bodily fluids including CSF and has been validated for detection of 8-F_2-IsoP in urine, plasma and other sample matrices. Briefly, each well contained 50 µl of sample or standard, 50 µl of 8-F_2-

IsoP AChE Tracer, and 50 µl of 8-F_2-IsoP Antiserum. Plates were incubated for 18 hours at 4°C. Wells were then washed 5 times with 200 µl of wash buffer at room temperature. Ellman's Reagent (200 µl) was added to each well. A Total Activity well contained only Ellman's Reagent and Tracer added just before the second incubation. A Reaction Blank contained only Ellman's Reagent, and a Non-Specific Binding well contained only Tracer, added before incubation, and Ellman's Reagent. Plates were covered and placed on an orbital shaker at 400rpm for 90 to 120 min at room temperature. The plate was then read on a Perkin Elmer Envision Multi-label Plate Reader at 405nm. Average replicates of each standard (0.08 pg/ml to 500 pg/ml) were used to generate the standard curve. CSF samples were analyzed in triplicate to confirm reproducibility

Statistical Analyses

Descriptive statistics were computed for sample characteristics (sociodemographics and leukemia risk category) and the baseline biomarker information (Table 2) as well as the longitudinal variables measured from Time 1 to Time 4 (Table 3).

To test the hypothesis that participants who had increased F2-isoprostanes and IL-8 biomarkers during the post-induction phase of treatment would experience higher severity of symptoms, longitudinal relationships were examined from the initiation of post-induction therapy (Time 1) to 4 and 8 months post-induction therapies (Times 2 & 3) to the beginning of maintenance/continuation therapy (Time 4), controlling for sociodemographics, leukemia risk category, and the baseline biomarkers. The longitudinal nature was expressed by the growth parameters—the initial status (or intercept) and the rate of change (or slope)—of the longitudinal variables.

More specifically, the longitudinal parallel process (LPP; Cheong et al., 2003), a process of two-step modeling, was utilized to test hypothesized longitudinal relationships. In the first step, the intercept and slope of each of the longitudinal variables were estimated separately by using univariate growth models with multilevel modeling in SAS Proc Mixed (SAS Institute, 2011). Such separate modeling helps not only manage the unbalanced, intermittent longitudinal

missing data across the different longitudinal variables, but also easily control for covariates, such as the sociodemographic and other baseline variables, in the estimation of the intercepts and slopes of the longitudinal variables so that the covariates would not need to be controlled again in the second step. Due to its modeling flexibility, LPP is increasingly reported in the literature for studies with longitudinal measurements (Dowling et al., 2015; Petrou et al., 2018; Sousa et al., 2014; Winkler et al., 2017; Yiotaldiotariotam et al., 2013).

In the second step, structural equation modeling was employed for testing the longitudinal relationships between the biomarkers and symptom cluster using IBM SPSS Amos (Arbuckle, 2010). The structural equation model fit was evaluated using the following model-fit indices: chi-square of the estimated model (χ^2), goodness of fit index (GFI), normed fit index (NFI), incremental fit index (IFI), relative fit index (RFI), comparative fit index (CFI), and root mean square error of approximation (RMSEA). A nonsignificant chi-square value ($p > .05$) suggests a good overall model fit to the data. For GFI, NFI, IFI, RFI, and CFI, values larger than .90 indicate that the model provides a good fit to the data, whereas RMSEA should be below .06. The fit indices and their criteria are commonly recommended in the literature (Hu and Bentler, 1999; Kline, 2005).

Results

Descriptive Statistics

Table 2 shows that among 218 participants in the study sample, the average age was 8.38 years (*SD* = 4.48). Sex was unbalanced with more boys than girls (56.0% vs. 44.0%); and race/ethnicity was even more unbalanced with nearly half being Hispanic (43.6%), 39.9% being non-Hispanic white, and much fewer being non-Hispanic black and non-Hispanic others (both 8.3%). As for leukemia risk category, 32.6% of the participants in the study sample were at very high risk, 21.6% at high risk, 34.9% at average or standard risk, and 11.0% at low risk. The

means of the two biomarkers at baseline were 8.38 (pg/ml) (*SD* = 10.42) for F2-isoprostanes and 142.77 (pg/ml) (*SD* = 112.20) for IL-8.

The means and standard deviations of the longitudinal variables across the four time points (Table 3) demonstrate that fatigue and pain decreased over time; whereas nausea remained almost the same from Time 1 to Time 3 before it decreased from Time 3 to Time 4. Both biomarkers greatly decreased from Time 1 to Time 2 and then remained almost the same for the remaining time points.

~~Because the three symptom measures (fatigue, pain, and nausea) were highly correlated with each other at each time point (Hockenberry et al., 2017), they were combined as a symptom cluster through exploratory factor analysis with maximum likelihood estimation which returned a one-factor solution with significant factor loadings from .35 to .88 and at least 54% of variance explained at each time point (Table 4).~~

Longitudinal Relationships

The model-fit indices for the initially hypothesized longitudinal relationships were satisfactory ($\chi^2(6) = 6.12$, $p = .409$; GFI = .99, NFI = .99, IFI = 1.00, RFI = .97, CFI = 1.00; and RMSEA = .010), but one path coefficient (i.e., from the intercept of F2-isoprostanes to the slope of symptom cluster) was not significant at the $\alpha = .10$ level. To obtain a parsimonious model, the non-significant path coefficient was removed in the final model. Thus, a final parsimonious model was reached as shown in Figure 2 with the standardized estimates of path coefficients. The model fit indices for the parsimonious model improved and all remained satisfactory ($\chi^2(7) = 6.13$, $p = .525$; GFI = .99, NFI = .99, IFI = 1.00, RFI = .97, CFI = 1.00; and RMSEA < .001).

From Figure 2, we can conclude that the positive, longitudinal relationships between biomarkers and symptom cluster did exist in the data. Specifically, patients with an initial high level of both biomarkers also experienced higher severity of symptoms initially ($\beta = .24$, $p < .001$ for F2-isoprostanes and $\beta = .21$, $p < .001$ for IL-8). For patients who had increased biomarkers

during the post-induction phase of treatment, they also experienced higher severity of symptoms over time (β = .14, p = .035 for F2-isoprostanes and β = .30, p = .015 for IL-8). In addition, for patients with an initial high level of IL-8, they experienced marginally higher severity of symptoms over time (β = .22, p = .082).

Discussion

Pain and fatigue levels were highest during the post-induction phase of treatment (Times 1 and 2), and then decreased slightly during maintenance therapy. This finding confirms that during the first few months of leukemia therapy, children experience the most intense symptoms. However, even at the later phase of treatment, children continued to experience symptoms. Nausea scores were in the mild range and relatively stable at all 4 measurement times consistent with the type of chemotherapy being administered for leukemia.

Increased levels of IL-8 and F2-isoprostanes in CSF samples were found, which suggests that inflammatory and oxidative stress pathways are activated in response to ALL treatment. As hypothesized, we also found that higher levels of these CSF biomarkers were associated with more severe symptoms. This study continues to support the influence of pediatric leukemia chemotherapy drugs on ROS production as by-products of cellular toxicity. Evidence supports that the oxidative stress pathway is interactive and may result in significant cellular toxicity. The current study's findings add to the evidence that activation of the oxidative stress pathway can induce numerous somatic symptoms; two previous studies specifically evaluating biomarkers for oxidative stress also confirmed activation of the oxidative stress pathway during childhood cancer treatment (Mazor et al., 2008; Papageorgiou et al., 2005).

These collective findings suggest that early increases in inflammatory and oxidative stress may be useful in identifying which children are at greater risk of experiencing treatment-related symptoms and in developing novel interventions to mitigate symptom severity. As

oxidative stress biological markers continue to be identified and described, it may be possible to determine individual susceptibility to oxidative stress and its influence on clinical outcomes and symptom severity in the future. As childhood cancer treatment continues to advance and more children are cured of their disease, findings from this work have the potential to allow individualized treatment focused on curing disease. Nursing interventions to minimize symptom toxicities can then be focused on the specific trajectories unique to each child with cancer.

It is worth noting that the study finding was based on only two biomarkers and three symptom measures. More biomarkers and more symptom measures would be interesting to be examined in future research for obtaining more robust results. In addition, the study finding was also based on a convenience sample from only four specific health systems. A larger randomized-cluster multi-site study on the topic in future research would be also desirable to provide more comprehensive evidence for informing future nursing research and practice.

Declaration of Conflicting Interests

The Authors declare that there is no conflict of interest.

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Figure Captions

Figure 1. Biochemical mechanisms and symptoms during childhood cancer treatment.

Figure 2. The final parsimonious model of the longitudinal relationships between biomarkers and symptom cluster in terms of their growth parameters (intercepts and slopes) with standardized estimates. ($^{\dagger}p < .10$. $^{*}p < .05$. $^{**}p < .01$. $^{***}p < .001$).

Table 1. Factor Loadings and Variance Explained in One-Factor Solution for Symptom Cluster at Each Time Point

Symptom	Factor—Symptom Cluster			
	Time 1	Time 2	Time 3	Time 4
Fatigue	.76	.71	.73	.88
Pain	.73	.64	.72	.49
Nausea	.53	.59	.56	.35
Variance Explained	63.2%	61.3%	63.2%	54.0%

Table 1. Symptom Measures

Symptom Instruments	Score Used in Data Analysis (Scale Range)	Severity Classification		
		Mild	Moderate	Severe
Fatigue	T-score of total			
Child Fatigue Scale (CFS)[a]	score (20-80)	≤ 28	29-37	≥ 38
Adolescent Fatigue Scale (FSA)[b]		≤ 35	36-55	≥ 56
Parent Fatigue Scale (PFS)[c]		≤ 39	40-56	≥ 56
Pain	Raw score (1-6)	≤ 2	3-4	≥ 5
Wong-Baker Faces Scale[d]				
Nausea	Raw score (0-100)	≤ 20	30-40	≥ 50
Visual Analog Scale (VAS)[e]				

[a]Hinds et al. (2010).

[b]Mandrell et al. (2011).

[c]Hockenberry et al. (2003).

[d]Wong and Baker (1988).

[e]Scott and Huskisson (1979) and Revill et al. (1976).

Table 2. Sample Characteristics and Baseline Biomarker Information (*N* = 218)

Characteristic		Mean / Frequency (*n*)	SD / Percent (%)
Age (*years*)		8.38	4.48
Sex	*Female*	96	44.0
	Male	122	56.0
Race/ Ethnicity	*Hispanic*	95	43.6
	Non-Hispanic White	87	39.9
	Non-Hispanic Black	18	8.3
	Non-Hispanic Other	18	8.3
Leukemia Risk Category	*Very High*	71	32.6
	High	47	21.6
	Average/Standard	76	34.9
	Low	24	11.0
Biomarkers at Baseline (*pg/ml*)	*F2-Isoprostanes*	8.38	10.42
	IL-8	142.77	112.20

Table 3. Means (*M*s) and Standard Deviations (*SD*s) of the Longitudinal Variables by Time (*N* = 218)

Variable (Scale Range)	Time 1			Time 2			Time 3			Time 4		
	n	***M***	***SD***	***n***	***M***	***SD***	***n***	***M***	***SD***	***n***	***M***	***SD***
Symptom Measures												
Fatigue (20-80)[a]	195	53.92	9.98	210	49.97	10.36	196	48.20	9.10	193	46.70	8.09
Pain (1-6)[b]	193	2.57	2.22	207	1.66	2.15	194	1.32	1.60	191	1.29	1.80
Nausea (0-100)[c]	192	14.33	21.53	206	14.06	21.43	192	15.52	21.35	192	12.05	19.14
Biomarkers (pg/ml)												
F2-Isoprostanes	195	7.49	8.77	209	6.36	5.17	196	6.58	7.71	194	6.66	8.42
IL-8	193	113.54	103.85	206	84.85	74.74	196	73.15	53.75	193	87.22	90.37

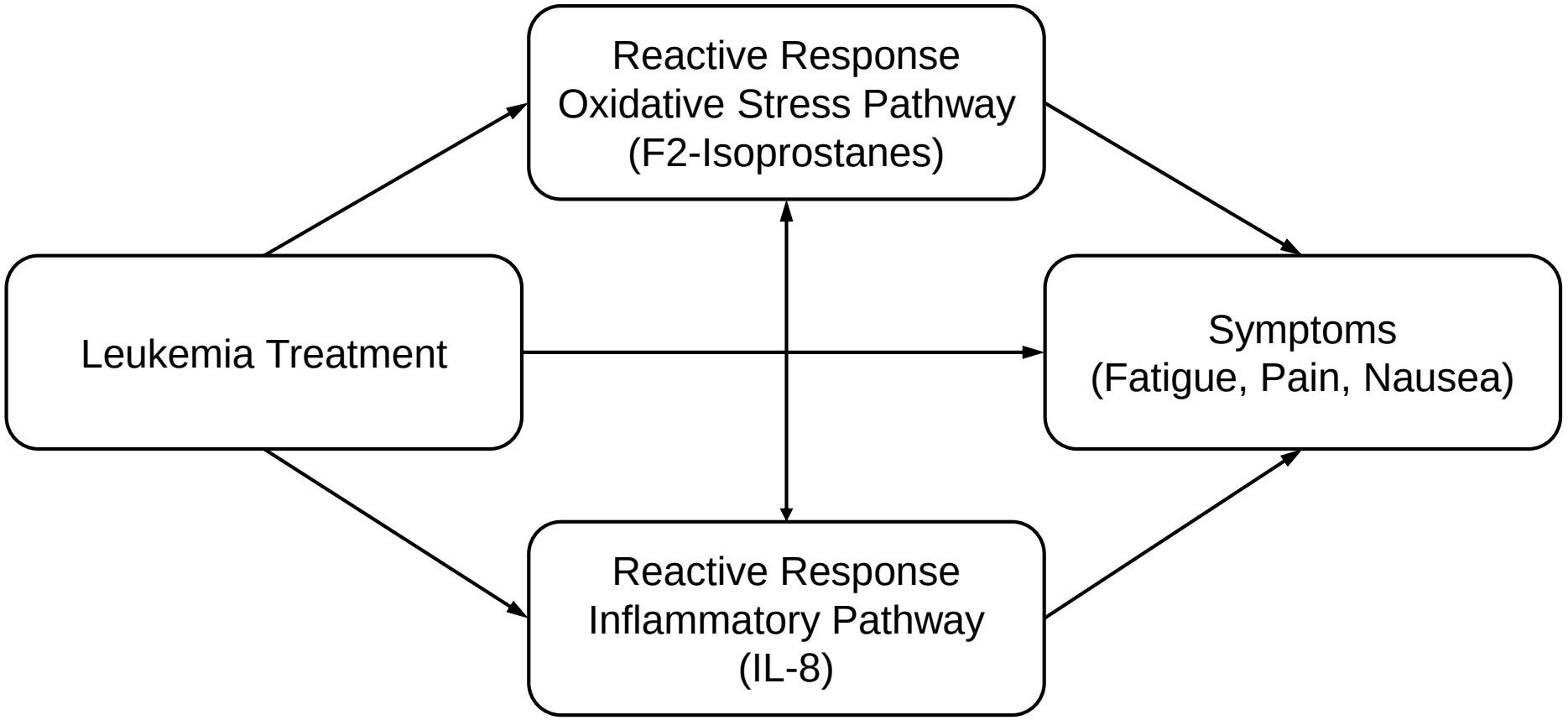
[a]Measured using the Child Fatigue Scale (CFS; Hinds et al., 2010), the Adolescent Fatigue Scale (FSA; Mandrell et al., 2011), and the Parent Fatigue Scale (PFS; Hockenberry et al., 2003). All the scores from the three instruments were converted to comparable, standardized T-scores.

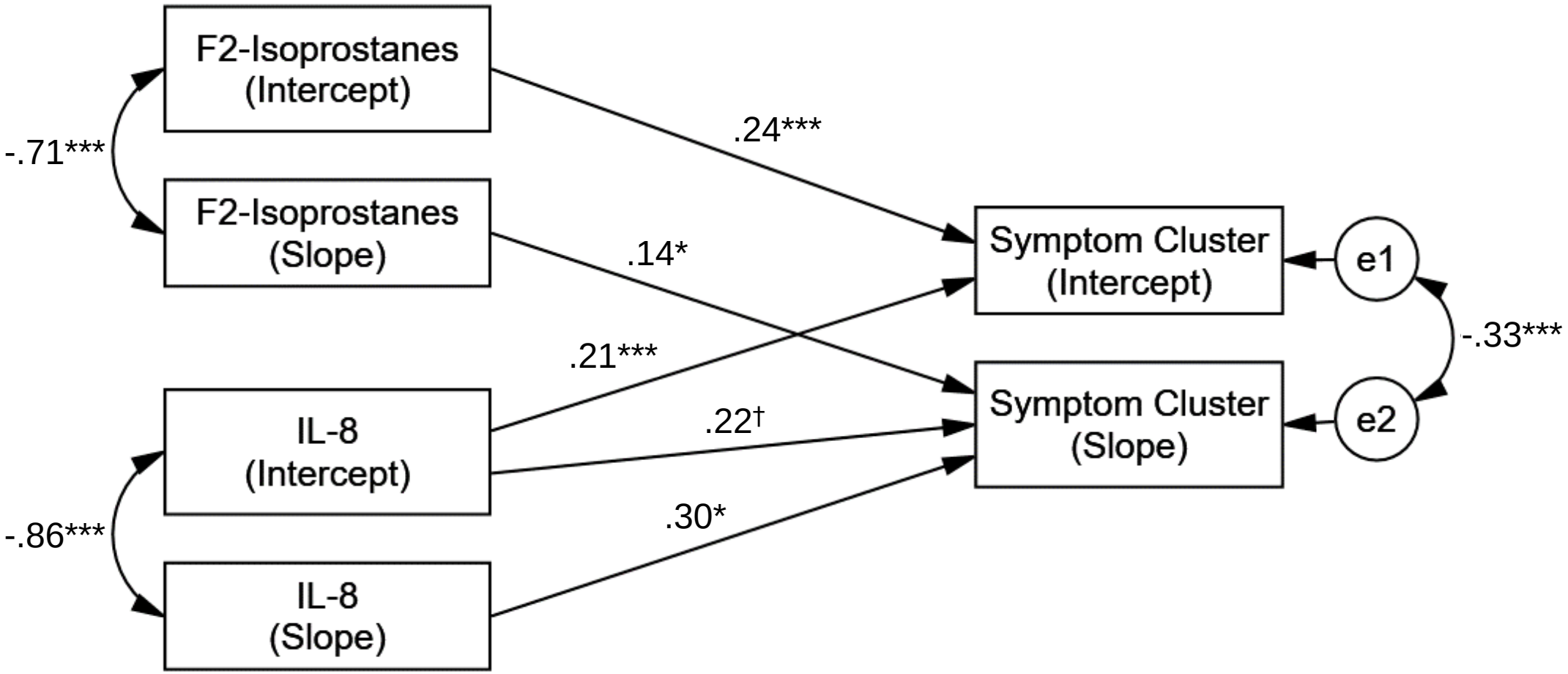
[b]Measured using the Wong-Baker Faces Scale (Wong & Baker, 1988).

[c]Measured using the Visual Analog Scale (VAS; Scott & Huskisson, 1979; Revill et al., 1976).

Table 4. Factor Loadings and Variance Explained in One-Factor Solution for Symptom Cluster at Each Time Point

Symptom	Factor—Symptom Cluster			
	Time 1	Time 2	Time 3	Time 4
Fatigue	.76	.71	.73	.88
Pain	.73	.64	.72	.49
Nausea	.53	.59	.56	.35
Variance Explained	63.2%	61.3%	63.2%	54.0%


Reactive Response
Oxidative Stress Pathway
(F2-Isoprostanes)
Leukemia Treatment
Symptoms
(Fatigue, Pain, Nausea)
Reactive Response
Inflammatory Pathway
(IL-8)


F2-Isoprostanes (Intercept)
F2-Isoprostanes (Slope)
IL-8 (Intercept)
IL-8 (Slope)
Symptom Cluster (Intercept)
Symptom Cluster (Slope)
e1
e2
-.71***
-.86***
.24***
.14*
.21***
.22†
.30*
-.33***