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Cytokine-associated fatigue prior to, during, and post-chemotherapy for breast cancer

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1. Introduction

Five-year breast cancer (BC) survival rates have increased dramatically over the past several decades (DeSantis et al., 2016). Yet, many women continue to report persistent adverse symptoms that they attribute to their breast cancer treatment; in particular the effects of chemotherapy (Dodd et al., 2010). Commonly reported symptoms include cognitive difficulties and fatigue that is often described as extreme exhaustion that interferes with daily activities (Abrahams et al., 2016; Bray et al., 2018; Manir et al., 2012; Yang and Hendrix, 2018). These symptoms are often not only reported during the chemotherapy period, but also long after chemotherapy has ended. Fatigue is a common symptom among women receiving chemotherapy for cancer (Lawrence et al., 2004). Chemotherapy-associated fatigue was reported by 91% patients in a cohort of patients with various forms of cancer, (Richardson and Ream, 1996). In another cohort of women who had undergone chemotherapy, radiation therapy, and other treatments with stage I-IV breast cancer, 83% reported symptoms of fatigue, 60% described their fatigue as problematic (Longman et al., 1996). In this cohort, 60% reported fatigue to be problematic. Fatigue of at least moderate severity is reported in 40–60% of BC survivors, and a meta-analysis of 27 studies (n = 12,327 cases) revealed that approximately 26% reported severe fatigue (Abrahams et al., 2016). In fact, the prevalence of reported fatigue is even greater than rates of reported cognitive deficits (Bray et al., 2018, Yang and Hendrix, 2018, Janelsins et al. Dutta, 2011). Difficulties with occupational and social functioning, including diminished productivity in daily activities, and reduced quality of life are often reported by BC survivors, which are attributed at least in part to these adverse symptoms following chemotherapy (Pullens et al., 2010; Schmidt et al., 2012).

Post-chemotherapy cognitive dysfunction has been the subject of considerable research given concern that chemotherapy may cause persistent cognitive deficits and brain dysfunction. While deficits on neuropsychological testing have been reported in a number of past studies of women receiving chemotherapy for BC (Janelsins et al., 2011; Ahles et al., 2012; Ahles and Saykin, 2002; Dutta, 2011; Jim et al., 2012; Vardy, 2009), the severity of deficits tends to be quite small, and are dependent on factors such as the age of the participants. Many of these studies were cross-sectional, so conclusions about change relative to pre-chemotherapy functioning cannot be reached. Those studies that were longitudinal have not consistently demonstrated clinically significant post-chemotherapy cognitive dysfunction (Jim et al., 2012; Lyon et al., 2016b). For example, in a longitudinal study of 77 BC survivors, we found that post-chemotherapy cognitive deficits were also present prior to treatment, and were associated with other clinical factors, including cancer-related factors, comorbidities (e.g., fatigue), and psychosocial influences (Lyon et al., 2016a, 2016b). The severity of cognitive deficits varied across the BC survivors and also as a function of the stage of treatment, though ultimately, chemotherapy did not produce severe long-term cognitive dysfunction, and only resulted in small reductions in cognitive performance during chemotherapy.

These findings raised obvious questions regarding the chemotherapy-associated cognitive deficits. Given that after two-years post-chemotherapy, most BC survivors did not exhibit significant objective cognitive dysfunction relative to pre-chemotherapy status suggested that other clinical factors were contributing to the subjective experience of cognitive difficulties. Fatigue seemed to be a logical candidate, as it is commonly reported in the context of chemotherapy, and can impact cognitive performance, and also peoples' self-perceptions of their performance. The high prevalence of fatigue relative to a lower prevalence of cognitive dysfunction in BC survivors suggests that subjective cognitive difficulties attributed chemotherapy may often be a manifestation fatigue rather than cognitive dysfunction per se among many BC survivors. Past studies have shown alterations of neural

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response on functional neuroimaging among BC survivors. These alterations varied relative to fatigue severity, cognitive status, and whether the assessment was done prior to, during or following chemotherapy, thereby linking chemotherapy, fatigue and cognition (Menning et al., 2015; Askren et al., 2014). BC survivors had functional neuroimaging alterations prior to, during, and following chemotherapy which varied relative to the severity of fatigue and subjective cognitive complaints.

To determine pathophysiological bases for cognitive symptoms among BC survivors, we previously examined the relationship between serum cytokine concentrations and cognitive performance (Lyon et al., 2016a). As hypothesized, elevated concentrations of proinflammatory serum cytokines were associated with cognitive deficits, a finding consistent with a growing body of research linking immune system dysfunction with cognitive dysfunction and other behavioral symptoms, including fatigue and sleep disturbance (Schmidt et al., 2012; Wood and Weymann, 2013; Menning et al., 2015; Dantzer and Kelley, 2007; Askren et al., 2014). Yet, the trajectory of cognitive and cytokine change across phases of the study (pre-, during, post-chemotherapy) did not mirror one another. The relationship between cognitive performance and cytokine concentrations was strongest prior to and following chemotherapy. During chemotherapy, cytokines were decoupled in relation to cognitive performance. Cognitive deficits prior to chemotherapy were apparently a manifestation of other clinical or psychosocial factors associated with BC (e.g., fatigue). Studies involving animal models provide evidence that systemic inflammatory response to cancer itself may contribute to behavioral alterations in tumor-bearing animals, independent of chemotherapy effects (Schrepf et al., 2015).

These findings suggest that among BC survivors, proinflammatory cytokines are associated with small reductions in cognitive performance, but also with symptoms of fatigue, which in turn contributes to the subjective experience of cognitive difficulties (i.e., chemobrain). Determining whether or not the relationship between serum cytokine concentrations, fatigue symptoms and cognitive performance change in a similar manner prior to, during, and following chemotherapy is a potentially important step towards achieving better understanding of these relationships. Serum cytokine concentrations have been shown to be associated with increased fatigue severity in a variety of medical conditions, including chronic fatigue syndrome (Cannon et al., 1997; Cannon et al., 1999; Moss et al., 1999). In the context of breast cancer, elevated cytokine concentrations have been shown associated with greater fatigue severity and functional impact (Bower, 2014; Bower, 2006; Bower et al., 2002; Bower et al., 2013; Bower et al., 2011b; Bower et al., 2014; Collado-Hidalgo et al., 2006; Collado-Hidalgo et al., 2008). Yet, the relationship between cytokine concentrations has not been consistent across studies. For example, when Cruz et al. (2015) examined changes in fatigue and cytokines after initiation of one round of chemotherapy relative to pre-chemotherapy, only plasma IL-1 concentrations differed between BC survivors with or without chemotherapy-associated fatigue. Of 43 BC survivors in this sample, 32 experienced significant increases in fatigue severity during chemotherapy. Yet none of the plasma cytokines were associated with these increases, and increased IL-1 concentrations occurred only among patients with severe fatigue. Notably, TGF-β concentrations analyzed from peripheral mononuclear cell fraction of mRNA was associated with greater fatigue, suggesting that the expression of cytokine genes and inflammatory pathways plays a role in the occurrence of symptoms of fatigue (Cruz et al., 2015).

The majority of past studies have been cross-sectional with comparison of BC survivors with or without severe fatigue, and many had relatively small sample sizes. None the less, they provide information regarding clinical factors that contribute to fatigue and elevations in cytokine concentrations. An interaction of group by time was observed in a study in which the impact of stress was examined between fatigued and non-fatigued survivors at two time points (Bower et al., 2007). Increased IL-6 levels increased in response to the stressor. In another study, increased fatigue and cytokine concentrations were observed following radiation therapy compared to baseline (Bower et al., 2009). Fatigue was also found to be associated with depressive symptoms and sleep disturbance, though these other two factors were not associated with cytokine concentrations (Bower et al., 2011b). In sum, there is strong evidence linking fatigue to elevated cytokine concentrations among BC survivors, a conclusion supported by systematic reviews of past research (Saligan and Kim, 2012, Bower Bower, 2014). However, whether this occurs primarily as a result of adverse chemotherapy effects remains an unresolved question. Determining the extent to which fatigue is a manifestation of cytokine alterations secondary to chemotherapy among BC survivors ultimately requires longitudinal studies that assess these and other clinical factors at multiple time points. By comparing the relationships between fatigue, cytokine concentrations, and other clinical factors (e.g., cognitive functions) prior to, during and long after chemotherapy, analysis of the trajectory of changes in the relationship among these factors as a function of treatment stage is possible.

Accordingly, the analyses in the current study were conducted to address this question; what are the effects of chemotherapy on pro-inflammatory cytokines and fatigue, along with cognition, and other clinical factors. The primary dependent measures of interest were Fatigue Severity, Fatigue Interference (the adverse impact of fatigue on everyday activities), and Worst Fatigue as reported by BC survivors from prior to chemotherapy until two years following chemotherapy. Changes in the relationship of plasma cytokine concentrations to these fatigue indices were examined, and compared to our previously reported findings regarding cytokines and cognitive performance. Evidence of a strong association between the response of pro-inflammatory cytokines and fatigue during chemotherapy would support the conclusion that the effects of chemotherapy have particular impact on this symptom, suggesting that chemotherapy-associated fatigue was likely attributable to elevated pro-inflammatory cytokine and immune system response. By comparing the relationship between cytokine concentrations and fatigue across treatment phases (pre-chemotherapy, chemotherapy, post-chemotherapy), it might be possible to determine the basis for coupling and decoupling of these factors in women with early-stage breast cancer prior to and during active chemotherapy and through two years survivorship.

2. Materials & methods

2.1. Study cohort

Details regarding the EPIGEN study cohort and methods have been described in our previous work(Lyon et al., 2016b; Lyon et al., 2016a; Starkweather et al., 2017). Participants were recruited from a designated National Cancer Center affiliated with the Medical Center of the Virginia Commonwealth University and four regional collaborative sites in Central Virginia. A total of 77 women with early-stage breast cancer, who ranged from 23 to 71 years of age, were ascertained and screened for eligibility. Inclusion criteria were as follows: (1) age of 21 years or older; and (2) a diagnosis of early-stage breast cancer (Stage I to IIIA) with a scheduled visit to receive chemotherapy. Exclusion criteria were as follows: (1) a previous history of cancer or chemotherapy; (2) a diagnosis of dementia; (3) active psychosis; or (4) a diagnosis of immune system disorders (e.g., multiple sclerosis, systemic lupus erythematosus). After providing informed consent (VCU IRB #HM 13194), participants were evaluated at five time points prior to chemotherapy through 2 years survivorship: before the start of chemotherapy (T1), at the midpoint chemotherapy (T2), 6 months after the initial chemotherapy (T3), 1 year after the initial chemotherapy (T4), and approximately 2 years following the initiation of chemotherapy (T5). The initial assessment (T1) was conducted after surgery, but prior to commencing chemotherapy in women receiving adjuvant therapy.

All procedures performed in this study were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

2.2. Measures

2.2.1. Clinical, demographic, and oncological variables

Demographic and disease profile information were collected by participant interview and medical record review. Demographic variables included age, race, marital status and educational level. Cancer-related variables included breast cancer stage (TNM) and hormone receptor status. Treatment-related variables included type of surgery, treatment regimen (adjuvant or neoadjuvant), chemotherapeutic regimen, radiation status, and hormonal agent status.

2.2.2. Fatigue

The Brief Fatigue Inventory (BFI) was used to assess Fatigue Severity and the extent to which fatigue was perceived to interfere with daily activities and quality of life (Mendoza et al., 1999). A measure of Worst Fatigue over the past 24 h was also analyzed. The BFI consists of nine items and each item is rated on an eleven-point scale (0 − 10), assessing physical, affective, cognitive, and social domains of fatigue. It has been shown to have strong reliability and validity (Cronbach's alpha = 0.95–0.96, internal consistency = 0.96), as well as correlation with severity ratings on other fatigue assessment inventories (Mendoza et al., 1999; Anderson et al., 2003; Hwang et al., 2003; Shafqat et al., 2005). Severity ratings of fatigue as determined by the BFI Overall Severity, Fatigue Interference, and Worst Fatigue scores were “None” (0), “Mild” (1–3), “Moderate” (4–6), and “Severe” (7 +) (Mendoza et al., 1999).

2.2.3. Cognitive function

Although cognition was not the primary focus of the current study, overall cognitive performance was assessed to provide a global cognitive index (GCI) which was entered along with the cytokines in statistical analyses conducted to examine the combined influence of cognition and cytokines on fatigue. A performance-based computerized neurocognitive testing system, CNS Vital Signs™ (CNSVS, https://www.cnsvs.com) was used to measure multiple neurocognitive domains, such as memory, psychomotor speed, reaction time, complex attention, and cognitive flexibility (Gualtieri and Johnson, 2006). Age-matched standard scores were derived from CNSVS, with higher scores indicating better neurocognitive performance. The subscales of the CNSVS were reported to have good test-retest reliability: attention ($r = 0.65$), memory ($r = 0.66$), psychomotor speed ($r = 0.88$), cognitive flexibility ($r = 0.71$), and reaction time ($r = 0.75$). This instrument has been used in women with breast cancer (Scherling et al., 2011; Breckenridge et al., 2012; Lasheen et al., 2017). It takes approximately 30 min to complete per session. While these measures were not the primary focus of the current analyses, we examined the combined contributions of fatigue and pro-inflammatory cytokine concentrations to cognitive performance in a secondary analysis. To do so, scaled scores for the attention, executive, processing speed, and memory domains were averaged to the GCI measure. These cognitive domains have been found to be most susceptible to the effects of chemotherapy and we used GCI previously in studies of chemotherapy effects on cognition (Lyon et al., 2016b; Lyon et al., 2016a).

2.2.4. Plasma cytokines

Phlebotomy was performed at each study visit from every participant. Whole blood was centrifuged, plasma separated, and then immediately aliquoted, frozen and stored in a − 80 °C freezer. A standard capture sandwich assay was used to determine the levels of different cytokines. Each captured antibody was coupled to a different bead set (Bio-Rad Laboratories, Hercules, California, USA) that uses a liquid suspension array of 17 sets of 5.5-μm beads (Bio-Plex Human Cytokine 17-plex panel) internally dyed with different ratios of two spectrally distinct fluorochromes to assign a unique spectral address. Each set of plasma beads was combined with a monoclonal antibody produced to be sensitive to specific cytokines/chemokines. Beads were incubated first (30 min to 2 h, at room temperature) with diluted standards (serial dilutions from 1.95 to 32, 000 pg/ml) and then with biotinylated detector antibodies (30 min, at room temperature). They were washed twice in phosphate-buffered saline, and incubated for 30 min at room temperature with phycoerythrin-conjugated streptavidin. Each measurement was taken in duplicate. Standard curves were generated by using the reference cytokine concentrations supplied by the manufacturer. Raw data (mean fluorescent intensity) were analyzed by Bio-Plex Manager Software (Bio-Rad Laboratories) to obtain concentration values. The lower limit of detection was < 10 pg/ml (based on detectable signal > 2 standard deviations (SD) above background). Samples were batch processed using plates from the same lots to reduce measurement variability. The panel of cytokines/chemokines consisted of interleukins (IL), including IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, and IL-17. Concentrations of Granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (G-CSF), interferon gamma (IFN-γ), monocyte chemotactic and activating factor (MCP-1), macrophage inflammatory protein (MIP-1β) and tumor necrosis factor (TNF-α) were also measured. Table 1 provides a list of cytokines/chemokines that were examined relative to the fatigue indices.

2.3. Statistical analyses

All analyses were conducted using SPSS v24. Descriptive statistics were obtained in the form of means, medians, and ranges for the continuous variables, and frequencies and percentages for the categorical variables. The temporal changes in the Fatigue Severity, Fatigue Interference, Worst Fatigue, global cognition, and cytokine concentrations across the five assessments (T1-T5) were tested using a linear mixed model repeated-measures analyses of variance (ANOVA) with Sidak adjustment for multiple comparisons. For these analyses, estimates of effects were derived via pairwise comparisons of T2-T5 relative to T1. T1 was selected as the reference time-point given that the long-term changes in cytokine concentrations and fatigue ratings resulting from the effects of chemotherapy were the critical question being addressed. The temporal pattern of cytokine concentrations and cognitive performance were also compared to previously reported findings. (Lyon et al., 2016b; Lyon et al., 2016a).

A two-stage analytic strategy was then employed to examine the relationship between cytokine concentrations and fatigue. In the first stage, the relationships between cytokine concentrations and ratings on the fatigue indices were examined at each of the five assessment time points (T1-T5) by hierarchical stepwise regression with backward removal of cytokines not contributing to the observed effect. In the second stage, demographic and clinical variables were entered as a second block into each hierarchical regression model to serve as independent measures relative to the fatigue indices. The rationale for this second hierarchical stage of regression of analysis was two-fold; 1) To determine whether associations between the cytokines and fatigue remained statistically significant when clinical variable may affect cytokine concentrations are considered, and 2) To determine the contribution of the cytokine concentrations beyond the relationships with fatigue accounted for by the BC-related clinical factors. The variables entered hierarchically as a block in the second stage are listed in Table 2.

A stepwise backward elimination regression approach was used to enable a determination of which cytokines were removed from the model at each step. In these analyses, the 17 cytokine concentrations were entered as the predictors in the model with Fatigue Severity, Fatigue Interference, and -Worst Fatigue treated as dependent measures in separate analyses. The a priori hypothesis was that certain cytokines

Table 1
Cytokines measured in the current study; immunological function and inflammatory action.

Pro-Inflammatory	
IL-1β	Produced by activated macrophages; mediates inflammatory response, cell proliferation, apoptosis; Induces pain via Cox-2 in CNS; Signals hypothalamus for thermoregulation (fever). Very elevated in acute infection; Moderate levels are adaptive.
IL-8	Produced by macrophages and also some endothelial and epithelial cells. Role in innate immunity. Major role in chemotaxis of neutrophils. Mediates inflammatory response and angiogensis.
IL-17	Pro-inflammatory, acting in conjunction with TNF-α and IL-1. Signalling of IL-17 implicated in various autoimmune diseases.
IFN-γ	Produces innate NK cells and acquired antigen specific cytotoxic response of certain T cells. Activates macrophages; critical for innate and adaptive immune response to pathogens, viral replication, and tumors.
TNF-α	Secreted by macrophages, monocytes and T-cells, neutrophils, and NK cells after stimulation with LPS. Also produced by astrocytes and microglia in CNS. Mediates systemic inflammation, inhibits viral replication, and tumorgensis.
MCP-1	CCL2 chemokine that induces monocyte attraction, degranulation of basophils and histimine release. Its reponse is induced by IL-1, TNF-α, and other cytokines.
MIP-1β	CCL4 chemokine. Produced by macrophages and generates local immunological response with superoxide production. Chemoattractant for many immune cells
Anti-Inflammatory	
IL-2	Role in microbial infection, and discrimination cell types ("non-self" vs. "self"). Mediates immunological response by binding to IL-2 receptors of lymphocytes. Often depleted in cancer. Used as a drug (Aldesleukin) in cancer treatment.
IL-4	Induces response of helper T-cellls. Involved in wound repair. Over expression in many forms of cancer.
IL-6	Secreted by T cells and macrophages. Triggers inflammaton, acute phase response; Implicated in systemic inflammation in multiple diseases and aging.
IL-10	Produced by monocytokes. Pleitropic cytokine. Has anti-inflammatory effects, inhibiting macrophages, dendritic cell function, and TNF-α. Acquires pro-inflammatory activity via IFN-γ stimulation.
IL-13	Involved in attenuation of monocyte and macrocyte function
G-CSF	A glycoprotein that stimulates bone marrow to produce stem cells and granuloccytes with release into blood circulation. Induces neurogenesis and neuroplasticity to counteract apoptosis. Is diminished in cancer and in the context of septsis. Supplemented in some cancer therapies.
Other	
IL-5	Stimulates B cell growth, increases immunoglobulin secretion (IgA), and is a key mediator in eosinophil activation.
IL-7	A hematopoietic growth factor secreted by stromal cells in the bone marrow and thymus, and also produced by dendritic cells, neurons, hepatocytes, and epithelial cells, but not produced by healthy lymphocytes. Supplemented in certain cancer chemotherapies.
IL-12	Produced by dendritic cells,[1] macrophages, neutrophils, and human B-lymphoblasts from antigenic stimulation.

Note: IL = Interleukin; INF-γ = Interferon-γ; MIP-1β = Macrophage Inflammatory Protein-1β; MCP-1 = Monocyte Chemoattractant Protein-1; TNFα = Tumor Necrosis Factor α; G-CSF = Granulocyte Colony Stimulating Factor.

(e.g., IL-1β, IL-6, IL-17, G-CSF, TNF-α) would be most strongly associated with Fatigue Severity, Fatigue Interference, and Worst Fatigue. However, in order to demonstrate a dissociation among cytokines affected in BC and its treatment, the larger panel of 17 cytokines were examined. A random intercept was included in the models to account for the within-subject correlation. To determine the relative contributions of cognitive functioning and cytokine concentrations to Fatigue Severity, Fatigue Interference, and Worst Fatigue, regression analyses were next conducted in which both the cytokines and cognitive scores were simultaneously entered as independent measures relative to the three dependent measures of fatigue on the day of the assessment.

3. Results

One hundred and fifty-four women were approached about study participation. Of these, 80 met study inclusion criteria and were consented for participation. Of the 80, three women (all Caucasian; ages 48, 62, and 66) failed to complete the multiple visits required for this longitudinal investigation, which resulted in a 96% retention rate. Two of the three women who did not complete the study elected to withdraw after T1 due to feeling "overwhelmed." The third woman, who developed osteomyelitis after T2, no longer met eligibility criteria and was excluded from further follow-up. Thus, a total of 77 women were included in the analysis.

3.1. Clinical characteristics

Demographic and clinical characteristics of the sample, including cancer- and treatment-related variables are provided in Table 2. All women in the cohort had been diagnosed with stage I to III breast cancer, the majority with a tumor grade of either 2 or 3. The most common tumor type was Luminal A (51%), though many were triple negative (29%) or estrogen/progesterone receptor negative (9%). Over half of the women were post-menopausal (51%). Most participants (91%) had surgical removal of their tumor prior to other treatment, while 9% had biopsy only. All participants were treated with chemotherapy following their baseline visit and 11% received neoadjuvant therapy. Hormonal therapy was given in 70% of cases (Table 3).

The Fatigue Severity ratings of the BC survivors averaged across the study assessments (T1-T5) indicated mild to moderate fatigue for the cohort as a whole, though this severity level is skewed by the fact that 32% reported no fatigue. Prior to chemotherapy at T1, 44% reported no fatigue, however at the initiation of chemotherapy (T2), the number of women reporting no fatigue dropped to 19%. Many participants reported overall moderate (30%) or severe (14%) fatigue, such that among those reporting any level of fatigue, over half describe their fatigue as moderate to severe (64%). Across all study time-points, a majority of BC survivors rated their worst level of Fatigue Severity over the previous 24 h as moderate to severe (52%). Among those reporting any level of fatigue, 75% rated their fatigue as moderate to severe. Many BC survivors also reported that fatigue was having a moderate to severe impact on the daily activities (e.g., Fatigue Interference; 29%).

3.2. Fatigue and cytokines: pre-chemotherapy to 24-month follow-up

Since overall mean severity ratings do not reflect the temporal dynamics of fatigue across chemotherapy phases or its functional impact over the two-year course of the study, the changes in fatigue indices were examined across the five visits. Fatigue severity varied significantly across the five assessments relative to the phase of chemotherapy intervention and subsequent follow-up (F (4, 306) = 28.0, $p < .001$). Participants reported lowest Fatigue Severity during the baseline assessment prior to chemotherapy, with an increase to its greatest severity at the chemotherapy mid-point (T2), and remained constant through the chemotherapy period. Following chemotherapy, fatigue had decreased by the 12- and 24-month follow-up visits. Pairwise comparisons across visits indicated that significant worsening of overall Fatigue Severity occurred between T1 and T2 ($p < .01$), and between T1 and T3 ($p < .01$), but that fatigue appears to be no different from baseline at T4 or T5, suggesting a chemotherapy-effect. Fatigue Severity The same temporal pattern was evident for ratings of worst fatigue over the prior 24-h and also for functional interference

Table 2
Demographic and clinical characteristics of study participants.

	Mean ± SD (n = 77)		
Age	51.5 ± 10.3		
Education	Frequency		
< 12th grade	09%		
12th grade	12%		
> 12th grade	79%		
Race			
African-American	29%		
Caucasian	71%		
Ethnicity			
Hispanic	04%		
Non-Hispanic	96%		
Clinical			
Tumor Type	Frequency	Chemotherapy	Frequency
Luminal A	51%	TAC	52%
Luminal B	11%	TC	28%
Triple Negative	29%	TCH	14%
HER2+, ER-, PR-	09%	CMF	03%
		AC	03%
		Neoadjuvant	11%
Grade		Hormonal Therapy	
1	07%	T4	26%
2	37%	T5	44%
3	56%		
Stage		Other Factors	
I	27%	Radiation	79%
IIA	41%	Post-menopause	57%
IIB	21%		
III	11%		
Surgery			
Biopsy	08%		
Lumpectomy	28%		
Segmental	20%		
Mastectomy	43%		

Note: Mean ± Standard Deviation are provided for age. Frequencies (%) are given for all other measures. TAC docetaxel (Taxotere) doxorubicin (Adriamycin), cyclophosphamide (Cytoxan); TC docetaxel (Taxotere), cyclophosphamide (Cytoxan); TCH docetaxel (Taxotere), carboplatin (Paraplatin), trastuzumab (Herceptin); CMF cyclophosphamide, methotrexate, fluorouracil; AC doxorubicin (Adriamycin), cyclophosphamide (Cytoxan).

attributed to fatigue.

Concentrations of six cytokines varied across the T1-T5 assessments [IL-6: F (4, 152) = 2.78, $p < .05$; IL-10: F (4, 152) = 2.42, $p < .05$; IL-12: F (4, 197) = 2.40, $p < .05$; IL-17: F (4, 171) = 4.04, $p < .01$; MCP-1: F (4, 204) = 3.71, $p < .01$; G-CSF: F (4, 212) = 3.42, $p = .01$]. However, the temporal pattern of change in cytokine concentration differed among these cytokines. For IL-6, moderately elevated concentrations were present prior to chemotherapy and there was actually a small but non-significant reduction at T2 with minimal change by the end of the chemotherapy period (T3), followed by a marked reduction in IL-6 concentrations at T4 and T5 to levels that was below those observed prior to chemotherapy. In contrast, IL-10, IL-12 and IL-17 were moderately elevated prior to chemotherapy, but then exhibited reduced concentrations during chemotherapy (T2-T3) and through the follow-up period (T4 and T5). This temporal course was also evident for G-CSF, which was most elevated prior to chemotherapy and then decreased subsequently such that by T5 very low concentrations existed. MCP-1 showed a very different temporal course, with moderate concentrations at T1 that increased and peaked at T3, but then remained stable at similar levels for T4-T5. Fig. 1 shows ratings of Fatigue Severity, Fatigue Interference, and Worst Fatigue at each assessment, along with CGI and concentrations of six cytokines (IL-6, IL-10, IL-12, IL-17, G-CSF, and MCP-1) over the two-year study period.

3.3. Cytokines and fatigue

Regression analyses were conducted at each chemotherapy phase (T1-T5), with the cytokines significantly associated with fatigue along with beta coefficients across at each of the five assessment indicated in Table 4 below.

At T1 (baseline), cytokine concentrations were significantly associated with Fatigue Severity ($R = 0.52$; F (6, 71) = 3.7, $p < .01$), Fatigue Interference ($R = 0.38$, F (4, 72) = 2.9, $p < .05$), and Worst Fatigue ($R = 0.50$; F (6, 71) = 3.34, $p < .01$). Six cytokines (IL-2, IL-6, IL-10. IL-12, MCP-1, TNF-α) were significantly associated with Fatigue Severity at baseline, with IL-6, IL-10, and TNF-α having a positive association, and IL-2, IL-12, MCP-1 having an inverse relationship. Three cytokines (IL-2, IL-6, IFN-γ) were associated with Fatigue Interference, with IL-6 and IFN-γ having a positive association, while IL-2 had a negative association. Six cytokines (IFN-γ, IL-2, IL-6, IL-10, IL-12, and MCP-1) were associated with Worst Fatigue, where IFN-γ, IL-6, and IL-10 had positive relationships and the remainder showed an inverse relationship.

During the onset of chemotherapy (T2), cytokine concentrations were significantly associated with Fatigue Severity ($R = 0.45$; F (3, 74) = 3.5, $p < .01$), Fatigue Interference ($R = 0.36$; F (3, 74) = 2.6, $p < .05$), and Worst Fatigue ($R = 0.49$; F (6, 71) = 3.71, $p < .01$). Again the direction of association varied by cytokine (See Table 1).

Immediately following chemotherapy completion (T3), the relationship between cytokine concentrations and fatigue again changed. Significant associations were found relative to Fatigue Severity ($R = 0.41$; F (3, 74) = 3.5, $p < .01$), Fatigue Interference ($R = 0.45$; F (3, 74) = 5.1, $p < .001$), and Worst Fatigue ($R = 0.37$; F (3, 74) = 3.56, $p < .05$). The cytokines associated with Fatigue Severity, Fatigue Interference, and Worst Fatigue included IL-6, IL-12, and MCP-1.

Cytokine concentrations were also associated with fatigue at T4 (12-month follow-up) for Fatigue Severity ($R = 0.28$; F (1, 76) = 5.6, $p < .05$) and Fatigue Interference ($R = 0.33$; F (2, 75) = 2.0, $p < .05$), but not for Worst Fatigue. The cytokines associated with fatigue at T4 included IL-17 related to Fatigue Severity, and IL-10 and IL-12 related to Fatigue Interference.

At the 24-month follow-up assessment (T5) three cytokines were associated with Fatigue Severity (R = 0.45; F (3, 74) = 5.4, $p < .01$), including IL-1β, IL-6, and TNF-α. These same three cytokines were associated with Fatigue Interference at T5 ($R = 0.46$; F (3, 74) = 4.3, $p < .01$). In addition to IL-1β and TNF-α, three additional cytokines were related to Worst Fatigue at T5 ($R = 0.49$; F (5, 72) = 3.86, $p < .01$), including G-CSF, IL-12, and MIP-1β.

3.4. Cognitive performance, fatigue, and cytokine concentrations

Overall cognitive performance for women in this study was in the average range (GCI = 109.5 + 16.8). Therefore, at a group level the BC survivors were not experiencing global cognitive dysfunction averaged across assessments. Further, as described in our prior work (Lyon et al., 2016b; Gullett et al., 2019) BC patients continued to improve cognitively over time, indicating they were benefiting from practice on the tasks similar to that of healthy adults. In analyses conducted to determine the associations of both cognitive functioning and cytokine concentrations to fatigue, a combination of cytokines and cognitive indices were significantly associated with both Fatigue Severity and Fatigue Interference at most assessments. Table 5 indicates the cytokines and cognitive associations with the fatigue indices from these analyses.

At T1, GCI was negatively associated with Fatigue Severity, along with three cytokines (IL-2, IL-6, IL-10). IL-6 and IL-10 had a positive association with Fatigue Severity (i.e., greater fatigue associated with greater cytokine concentrations), whereas IL-2 showed the opposite relationship ($R = 0.50$; F (4, 73) = 4.5, $p < .01$). With respect to

Table 3
Longitudinal change in fatigue and cytokines over time ($N = 77$).

Fatigue measure	T1	T2	T3	T4	T5	Overall
	(Baseline)	(4 weeks)	(6 months)	(1 year)	(2 years)	
Severity Overall (mean)	2.39	3.88**	3.54**	2.85	3.00	
None	44.1%	18.8%	27.1%	32.8%	35.4%	31.6%
Mild	22.1%	24.8%	24.5%	26.6%	22.9%	24.2%
Moderate	20.8%	42.1%	29.2%	30.8%	27.3%	30.0%
Severe	13.0%	14.3%	19.2%	9.8%	14.4%	14.1%
Interference (mean)	1.76	3.10**	2.52*	1.94	2.05	
None	53.2%	28.5%	31.4%	49.3%	44.3%	41.3%
Mild	22.1%	29.9%	38.3%	24.1%	34.5%	29.8%
Moderate	16.9%	26.0%	15.1%	16.8%	14.2%	17.8%
Severe	7.8%	15.6%	15.2%	9.8%	7.0%	11.1%
Worst (mean)	2.92	4.61**	4.29**	3.59	3.67	
None	44.1%	19.8%	25.7%	33.9%	33.6%	31.4%
Mild	16.9%	14.5%	14.9%	16.9%	18.8%	16.4%
Moderate	18.2%	32.9%	27.1%	23.9%	23.1%	25.0%
Severe	20.8%	32.8%	32.5%	25.3%	24.5%	27.2%
Cytokine	T1	T2	T3	T4	T5	Overall
	(baseline)	(4 weeks)	(6 months)	(1 year)	(2 years)	
	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	Mean (SE)	F-statistic
IL-6	23.6 (11.8)	30.6 (8.8)	42.6 (13.3)	47.5 (37.2)	7.2 (1.2)†	3.48*
IL-10	64.9 (42.4)	72.4 (34.1)†	83.2 (45.6)	143.7 (127.4)*	15.5 (127.4)*	1.56
IL-12	92.2 (53.6)	116.9 (60.9)	243.9 (172.2)†	179.8 (142.3)	32.4 (11.1)**	2.19†
IL-17	90.5 (44.1)	63.3 (29.8)	127.6 (55.1)	74.4 (32.3)*	74.8 (45.9)**	4.59**
G-CSF	28.6 (5.5)	25.6 (4.1)	24.7 (6.1)	75.4 (10.4)†	19.9 (5.7)*	2.97*
MCP-1	51.9 (8.3)	61.9 (8.7)	100.8 (15.0)**	101.1 (22.7)**	74.2 (9.15)**	5.15**

†Difference compared to baseline (T1) at $0.10 > p > .05$; * Significant difference compared to baseline (T1) at $p < .05$; ** Significant difference compared to baseline (T1) at $p < .01$.
Note: IL = Interleukin; MCP-1 = Monocyte Chemoattractant Protein-1; G-CSF = Granulocyte Colony Stimulating Factor.

Fatigue Interference at T1, only GCI was retained as a significant correlate ($R = 0.31$; $F(1, 76) = 7.5$, $p < .01$). GCI was inversely related to Worst Fatigue at T1, along with two cytokines (IL-2, IL-6) (R = 0.50; F (3, 74) = 4.65, $p < .01$).

At T2 and T3 (during and immediately after chemotherapy), GCI was not retained and only the cytokines related to Fatigue Severity and Fatigue Interference were retained in the regression model (see Table 4). However, for Worst Fatigue, GCI was retained in the model at T2 along with two cytokines (IL-8, IL-17) ($R = 0.43$; F (3, 74) = 3.82, $p < .01$). At T3, while the entire model was trending for significance ($R = 0.29$; F (1, 76) = 3.18, $p = .053$), and GCI was not retained in the model, one cytokine (IL-5) was retained as related to Worst Fatigue.

At T4 (12-month follow-up), only one cytokine (IL-17) and the GCI were negatively associated with Fatigue Severity ($R = 0.41$; F (2, 75) = 12.3, $p < .01$). For Fatigue Interference, the model was overall significant ($R = 0.39$; F (1, 76) = 3.8, $p < .05$), and only GCI was retained in the model at T4. Worst Fatigue was overall significant ($R = 0.44$; F (3, 74) = 3.97, $p < .01$) and related to two cytokines (MCP-1, MIP-1β) as well as GCI.

By T5, multiple cytokines along with GCI were significantly associated with Fatigue Severity ($R = 0.63$; F (7, 70) = 5.7, $p < .001$), Fatigue Interference (R = 0.63; F (5, 62) = 8.6, $p < .001$), and Worst Fatigue ($R = 0.60$; F (6, 71) = 3.97, $p < .01$). Six cytokines (IL-1β, IL-4, IL-6, IL-12, TNF-α, G-CSF) along with GCI were associated with Fatigue Severity at T5. Three of these cytokines (IL-1β, IL-6, TNF-α) along with GCI were associated with Fatigue Interference at T5, and five cytokines (IL-4, IL-8, IL-13, MCP-1, TNF-α) along with CGI were associated with Worst Fatigue at T5.

3.4.1. Influence of the clinical covariates

When adding clinical covariates into the model, the cytokines retained in the regression models were relatively consistent with those associated with various measures of fatigue discussed previously and presented in Table 4. Inclusion of the clinical covariates into the models generally strengthened the overall associations with the three fatigue indices (see Table 6).

At T1, five cytokines (IL-2, IL- 6, IL-10, IL-12, and MCP-1) and one clinical factor (HER positivity) were significantly associated with overall Fatigue Severity ($R = 0.56$; $F(6, 71) = 3.44$, $p < .01$). None of the cytokines or clinical factors were significantly associated with Fatigue Interference at T1 ($p = .08$), though HER positivity approached significance. Five cytokines (IL-2, IL- 6, IL-10, IL-17, and MCP-1) and no clinical factors were associated with Worst Fatigue ($R = 0.46$; F (5, 72) = 3.10, $p < .01$).

At T2, three cytokines (IL-7, IL-12 and IFN-γ) and one clinical factor (progesterone receptor positivity) were retained as significantly associated with Fatigue Severity ($R = 0.48$; F (4, 73) = 4.10, $p < .01$). Three cytokines (IL-1β, IL-7, TNF-α) and one clinical factor (progesterone receptor positivity) were retained in the model as associated with Fatigue Interference at T2 (R = 0.48; F (4, 73) = 3.43, $p < .01$). Six cytokines (G-CSF, IFN-γ, IL-2, IL-7, IL-12, TNF-α) and no clinical factors were significantly associated with Worst Fatigue at T2 ($R = 0.47$; $F(6, 71) = 3.87$, $p < .001$).

At T3, one cytokine (IL-17) and one clinical factor (Race) were significantly associated with Fatigue Severity (R = 0.48; F (2, 75) = 5.06, $p < .01$). Three cytokines (IL-6, IL-12, MCP-1) were associated with Fatigue Interference, but no clinical factors were retained ($R = 0.53$; F (3, 74) = 4.95, $p < .01$). One cytokine (IL-1β) and no clinical factors were associated with Worst Fatigue ($R = 0.44$; F (1, 76) = 3.14, $p < .05$).

At T4, only IL-17 was significantly associated with Fatigue Severity, with no clinical factors retained in the model ($R = 0.36$; F (1, 76) = 4.90, $p < .05$). A weaker relationship was observed relative to Fatigue Interference as only the clinical variable of treatment with neoadjuvant therapy was significantly associated with this fatigue measure ($R = 0.27$; F (1, 76) = 5.40, $p < .05$). Similarly, only one clinical factor (Progesterone Receptor Positivity) was retained as it relates to Worst Fatigue at T4 ($R = 0.25$, F (1, 76) = 4.59, $p < .05$).

By two-years post-chemotherapy (T5), the association of the chemokines and clinical factors to Fatigue Severity was stronger ($R = 0.58$,

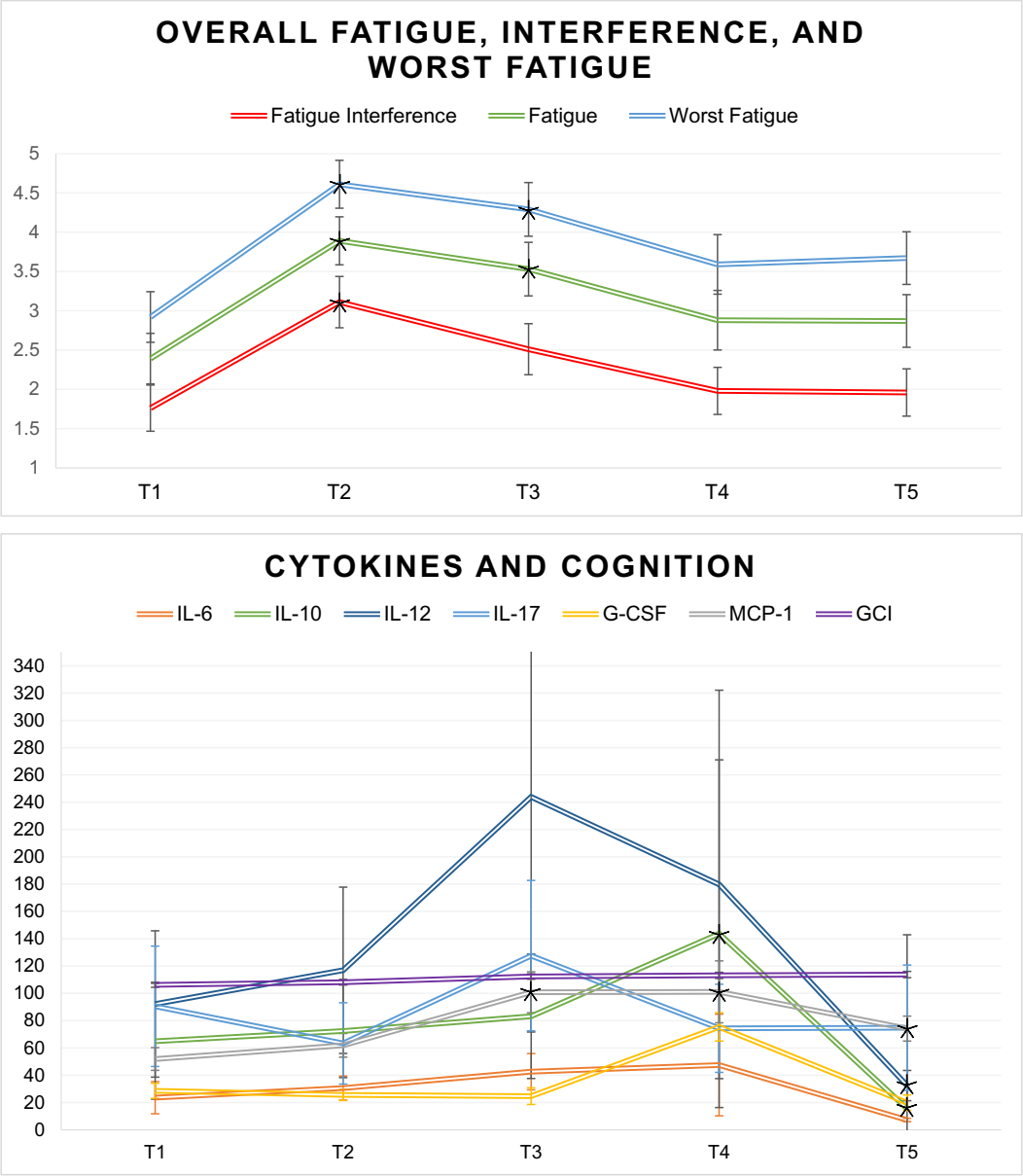

Fig. 1. Fatigue, fatigue interference, cytokines, and cognition as a function of study visit. *Note:* GCI = Global Cognition Index standard score with a mean of 100 and standard deviation of 15; T1 = pre-chemotherapy; T2 = mid-point chemotherapy; T3 = end of chemo-therapy; T4 = six months follow-up; T5 = two-year follow-up; Cytokine values are in pg/mL; Cancer-related Fatigue, Fatigue Interference, and Worst Fatigue scores presented as a function of mean raw score on the Brief Fatigue Inventory (BFI). Indicates change from baseline (T1) at $p < .05$.

F (6, 70) = 4,43, $p < .01$). Neoadjuvant therapy was significantly associated with Fatigue Severity, along with five cytokines (G-CSF, IL-1β, IL-4, IL-12, TNF-α). A strong association was also evident for Fatigue Interference at T5 ($R = 0.66$, F (3, 74) = 6.46, $p < .001$) with two cytokines (IL-6, TNF-α) and one clinical variable (Neoadjuvant therapy) retained in the model. This was also the case for Worst Fatigue ($R = 0.56$, F (3, 74) = 4.65, p < .01), where neoadjuvant therapy again retained as significant along with two cytokines (TNF-α, MIP-1β).

3.4.2. *Influence of cytokines, cognition, and clinical variables*

When considering the entirety of data, including all cytokines, clinical variables, and cognitive performance, a similar pattern emerges with some important changes over prior models (Table 7). At T1, several cytokines were related to fatigue, where IL-2, IL-6, and IL-10 were most commonly related to the three fatigue outcomes, along with menstruation status and cognitive functioning. At T2, progesterone receptor positivity was retained as a significant clinical predictor of all three fatigue ratings, while cognition was only predictive of overall fatigue severity, with IL-7 and IL-12 being the most common cytokines associated with fatigue at this time point. At the study time point immediately following the completion of chemotherapy (T3), cognition was once again predictive of overall fatigue severity, and IL-12 was the cytokine most commonly associated with the fatigue outcome measures. At the 12-month follow-up (T4), IL-17 was associated with overall Fatigue Severity, while cognition was a consistent predictor of all three fatigue measures. Lastly, at the 24-month follow-up time point, cognitive functioning performance in addition to numerous cytokines re-emerged as related to fatigue, most commonly G-CSF, IL-1β, and TNF-α, among others (Table 7).

Table 4
Cytokines associated with fatigue severity and interference at each study phase (T1-T5) (N = 77).

	T1			T2			T3			T4			T5		
	Model	β	R	Model	β	R	Model	β	R	Model	β	R	Model	β	R
Fatigue Severity	IL-2**	−0.57	0.52**	IL-7**	0.54	0.45**	IL-6*	0.32	0.41**	IL-17*	−0.28	0.28*	IL-1β**	−0.62	0.45**
	IL-6*	0.36		IL-12*	−0.31		IL-12*	−0.31					IL-6*	0.41	
	IL-10*	0.44		TNF-α*	0.31		MCP-1*	−0.41					TNF-α*	0.36	
	IL-12*	−0.24													
	MCP-1*	−0.43													
	TNF-α*	0.22													
Fatigue Interference	IFN-γ*	0.38	0.38*	IL-7*	0.44	0.36*	IL-6*	0.34	0.49**	IL-12*	0.29	0.33*	IL-1β**	−0.57	0.46**
	IL-2*	−0.35		IL-12*	−0.29		IL-12**	−0.46		IL-10*	−0.24		IL-6*	0.41	
	IL-6*	0.23		TNF-α*	0.29		MCP-1*	−0.38					TNF-α*	0.37	
Worst Fatigue	IFN-γ*	0.35	0.50**	G-CSF*	−0.34	0.49**	IL-6*	0.36	0.37*	NS		0.03	G-CSF**	−0.43	0.49**
	IL-2**	−0.75		IFN-γ**	−0.65		IL-12*	−0.41					IL-12*	0.39	
	IL-6*	0.47		IL-2*	0.53		MCP-1*	−0.27					IL-1β*	−0.35	
	IL-10**	0.45		IL-7**	0.43								MIP-1β*	0.32	
	IL-12*	−0.31		IL-12*	−0.32								TNF-α*	0.36	
	MCP-1**	−0.36		TNF-α*	0.35										

Note: IL = Interleukin; MIP-1β = Macrophage Inflammatory Protein-1β; MCP-1 = Monocyte Chemoattractant Protein-1; TNFα = Tumor Necrosis Factor α; G-CSF = Granulocyte Colony Stimulating Factor; β = Standardized Beta Weight.
* $p < .05$.
** $p < .01$.

4. Discussion

Serum cytokine concentrations were associated with symptoms of fatigue among BC survivors and both varied over the two-year time course of this prospective longitudinal study. With respect to this relationship, pro-inflammatory cytokines were associated with greater fatigue, and anti-inflammatory cytokines with less fatigue: 1) Symptoms of fatigue were common, though not ubiquitous among BC survivors; 2) Among individuals who indicated that they were experiencing fatigue, a significant proportion reported their symptoms of fatigue to be moderate to severe, particularly the worst fatigue over the past 24-h; 3) Fatigue symptoms were perceived to have an adverse functional impact, as many BC survivors indicated that it interfered with daily activities; 4) Fatigue symptoms peaked during chemotherapy, but then improved over two years following chemotherapy. Serum cytokine concentrations followed a similar trajectory as fatigue: 5) Pro-inflammatory cytokine concentrations also peaked during chemotherapy, most of which were reduced at the the 24-month follow-up compared to both chemotherapy and pre-cheomotherapy levels. 6) The relationship between cytokine concentrations and fatigue symptoms varied as a function of treatment phase. 7) Prior to chemotherapy, cognitive function contributed along with the cytokines to fatigue severity, whereas during chemotherapy cognitive performance was no longer associated with fatigue. 8) The association between the cytokines and fatigue symptoms was evident even when clinical factors, including oncological status, were considered simultaneously.

The current findings support and extend the results from past studies of fatigue associated with chemotherapy in breast and other types of cancer conducted by ours and other research groups(Saligan et al., 2015; Bower, 2014; Bower and Lamkin, 2013; Bower, 2005). We have previously shown that the severity of reported fatigue symptoms was greater than the severity of cognitive dyfunction in this cohort of BC

Table 5
Cytokines and overall cognitive performance (GCI) associated with fatigue severity and interference at each study phase (T1-T5) (*N* = 77).

	T1			T2			T3			T4			T5		
	Model	β	R	Model	β	R	Model	β	R	Model	β	R	Model	β	R
Fatigue Severity	IL-2*	−0.43	0.50**	IL-7*	0.40	0.48**	IL-6*	0.36	0.45**	IL-17*	−0.23	0.41**	IL-1β**	−0.84	0.63**
	IL-6*	0.41		IL-12*	−0.40		IL-12*	−0.42		GCI*	−0.31		IL-4*	0.42	
	IL-10*	0.37		TNFα†	0.27		MCP-1*	−0.32					IL-6*	0.33	
	GCI*	−0.31		GCI	NS		GCI	NS					IL-12*	0.36	
													TNFα*	0.36	
													G-CSF*	−0.43	
													GCI*	−0.33	
Fatigue Interference	GCI*	−0.31	0.31*	IL-6*	0.30	0.48**	IL-6*	0.36	0.50**	GCI*	−0.39	0.39*	IL-1β**	−0.53	0.63**
				IL-7*	0.43		IL-12*	−38					IL-6*	0.36	
				IL-12*	−0.33		MCP-1*	−0.34					TNFα*	0.32	
				TNFα*	0.37		GCI	NS					GCI*	−0.42	
				GCI	NS										
Worst Fatigue	IL-2*	−0.30	0.50**	IL-8**	−0.60	0.43**	IL-5*	−0.25	0.29†	MCP-1*	−0.31	0.44**	IL-4*	−0.33	0.60**
	IL-6*	0.33		IL-17**	0.56		GCI	NS		MIP-1β*	−0.33		IL-8*	−0.34	
	GCI**	−0.39		GCI**	−0.38					GCI**	−0.34		IL-13*	0.34	
													MCP-1**	−0.46	
													TNFα*	0.30	
													GCI**	−0.44	

Note: GCI = Global Cognitive Index; Note: IL = Interleukin; MIP-1β = Macrophage Inflammatory Protein-1β; MCP-1 = Monocyte Chemoattractant Protein-1; TNFα = Tumor Necrosis Factor α; G-CSF = Granulocyte Colony Stimulating Factor; β = Standardized Beta Weight.
* $p < .05$.
** $p < .01$.

Table 6
Cytokines and clinical variables associated with fatigue severity and interference at each study phase (T1-T5) (N = 77).

	T1			T2			T3			T4			T5		
	Model	β	R	Model	β	R	Model	β	R	Model	β	R	Model	β	R
Fatigue Severity			0.56**			0.48**			0.48**			0.36*			0.58**
	IL-2**	−0.74		IL-7**	0.49		IL-17**	−0.49		IL-17*	−0.26		G-CSF*	−0.43	
	IL-6**	0.52		IL-12*	−0.37								IL-1β**	−0.59	
	IL-10*	0.44		IFN-γ*	−0.34								IL-4*	0.49	
	IL-12*	−0.40											IL-12*	0.39	
	MCP-1**	−0.43											TNF- α*	0.35	
							Race*	−0.24							
	HER pos.*	0.24		Progesterone R.P.**	−0.30		Progesterone R.P*	−0.26					Neoadjuvant*	0.24	
Fatigue Interference			0.29†			0.48**			0.53**			0.27*			0.66**
	HER pos.†	0.22		IL-1β*	−0.34		IL-6*	0.43					IL-6**	0.62	
				IL-7**	0.37		IL-12**	−0.68					TNF- α**	0.45	
				TNF- α*	0.41		MCP-1*	−0.33							
				Progesterone R.P.**	−0.45					Neoadjuvant*	0.27		Neoadjuvant**	0.40	
Worst Fatigue			0.46**			0.53			0.44*			0.25*			0.56
	IL-2**	−0.57		G CSF*	−0.32		IL-1b*	−0.43					MIP-1β**	0.39	
	IL-6**	0.54		IFN-γ**	−0.70								TNF- α*	0.41	
	IL-10**	0.54		IL-2*	0.55										
	IL-12*	−0.40		IL-7**	0.47										
	MCP-1*	−0.35		IL-12*	−0.32										
				TNF-α**	0.38					Progesterone R.P.*	−0.25		Neoadjuvant**	0.33	

Note: Progesterone R.P. = Progesterone Receptor Positivity; HER-pos = Human Epidermal Growth Factor Receptor Positivity; Note: IL = Interleukin; MIP-1β = Macrophage Inflammatory Protein-1β; MCP-1 = Monocyte Chemoattractant Protein-1; TNFα = Tumor Necrosis Factor α; G-CSF = Granulocyte Colony Stimulating Factor; INF-γ = Interferon-γ.

† $0.10 < p > .05$.

* $p < .05$.

** $p < .01$

Table 7
Cytokines, cognition, and clinical variables associated with fatigue severity and interference at each study phase (T1-T5) (N = 77).

	T1			T2			T3			T4			T5		
	Model	β	R	Model	β	R	Model	β	R	Model	β	R	Model	β	R
Fatigue Severity			0.66**			0.52**			0.55**			0.41**			0.66**
	IL-2**	−0.71		IL-7**	0.39		IL-12*	−0.34		IL-17*	−0.24		G-CSF*	−0.42	
	IL-6*	0.42		IL-12*	−0.36		MCP-1*	−0.32					IL-1β**	−0.65	
	IL-10**	0.54											IL-4*	0.52	
	IL-12*	−0.39											IL-12**	0.54	
	MCP-1**	−0.36													
	TNF- α*	0.32													
	Menstr. Status**	0.41		Progesterone R.P.*	−0.37		Age*	−0.26							
	GCI**	−0.30		GCI*	−0.30		GCI**	−0.30		GCI*	−0.30		GCI**	−0.47	
Fatigue Interference			0.63**			0.48**			0.49**			0.42**			0.72**
	IFN-γ*	0.45		IL-1β*	−0.33		IL-6*^	0.46					G-CSF*	−0.31	
	IL-2**	−0.66		IL-7*	0.37		IL-12**	−0.54					IL-1β**	−0.65	
	IL-6*	0.33		TNF- α*	0.42		MCP-1*	−0.27					IL-6**	0.44	
	IL-7*	−0.34											TNF- α*	0.32	
	IL-10*	0.35													
	IL-17*	−0.38													
	Age**	−0.68													
	Menstr. Status**	0.50		Progesterone R.P.*	−0.44								Neoadjuvant**	0.27	
	GCI**	−0.35								GCI**	−0.33		GCI**	−0.38	
Worst Fatigue			0.63**			0.53**			0.39*			0.30*			0.65**
	IL-2**	−0.57		G CSF*	−0.33		IL-12*	−0.27					G-CSF*	−0.30	
	IL-6**	0.54		IFN-γ**	−0.68								IL-1β*	−0.42	
	IL-7**			IL-2*	0.52								IL-12*	0.39	
	IL-10**	0.54		IL-7**	0.48								MIP-1β*	0.31	
	IL-12*	−0.40		IL-12*	−0.34								TNF- α*	0.37	
	IL-13*	−0.35		TNF-α**	0.38										
	Age**	−0.55													
	Menstr. Status*	0.41		Progesterone R.P.*	−0.22										
	GCI**	−0.34								GCI*	−0.30		GCI**	−0.32	

Note: Progesterone R.P. = Progesterone Receptor Positivity; HER-pos = Human Epidermal Growth Factor Receptor Positivity; Menstr. Status = Menstrual Status pre- or post-menopausal.

* $p < .05$.

** $p < .01$.

survivors (Gullett et al., 2019). Fatigue was also among clinical factors that contributed to cognitive deficits among some these individuals (Lyon et al., 2016b). Ultimately, symptoms of fatigue and cognitive performance co-occur (Gullett et al., 2019) and appear to be functionally related. Serum cytokines are associated with cognitive deficits among BC survivors (Lyon et al., 2016a), as they are with fatigue symptoms in the current study. While the common occurrence of fatigue symptoms among cancer survivors and their relationship to cytokines has been demonstated in a number of other past studies (Saligan et al., 2015; Bower, 2014; Bower and Lamkin, 2013), many of these studies were cross-sectional and/or had relatively small samples sizes. Longitudinal studies adddressing this topic have yielded mixed results. Evidence indicating that fatigue symptoms remained for a long period of time following chemotherapy was shown in an early study of BC survivors (Bower et al., 2006). Yet the fact in another study no cytokines was associated with increases in fatigue during chemotherapy relative to pre-chemotherapy raised question about the extent to which role of inflammatory cytokines were responsible for fatigue symptoms (Cruz et al., 2015). On the other hand, the current findings linking cytokines, chemotherapy and fatigue provide evidence that inflammatory processes do in fact conribute to symptoms of fatigue. These relationships are further supported by studies of gene expression in BC survivors undergoing chemotherapy which show that the expression of cytokine genes and pathways are associated with fatigue symptoms (Kober et al., 2016; Bower et al., 2011a; de Alcantara et al., 2019). However, results from the current study also suggest that chemotherapy is not the sole determinant of the relationship between fatigue and cytokines given that fatigue was reported by a significant proportion of BC survivors prior to chemotherapy, and cytokine concentrations were associated with fatigue severity and functional interference. This may be one reason for the lack of an association between cytokine concentrations and fatigue during chemotherapy reported by Cruz et al. (2015). Examination of the trajectory of fatigue symptoms, cognitive performance, cytokine concentrations, and other clinical factors, provides clear illustration of this.

Even before chemotherapy was initiated (T1), mild levels of Fatigue Severity and Fatigue Interference were reported by the BC survivors based on published BFI interpretative criteria (Mendoza et al., 1999). Yet, this here was considerable variance with respect to Fatigue Severity and Fatigue Interference. Over 40% of the BC survivors reported no baseline fatigue prior to chemotherapy, though the remainder reported fatigue ranging from mild to severe. Cytokine concentrations were also associated with fatigue prior to chemotherapy. This finding has potential clinical significance as it provides further evidence that symptoms often attributed to chemotherapy are actually present prior to treatment, and that fatigue can actually be linked to biological factors linked to inflammation and ultimately immune responsivity. Presumably, the activity of cytokines prior to chemotherapy is in response to cancer itself. The fact that concentrations of these same cytokines also varied with the extent to which fatigue interfered with daily activities reinforces the potential clinical relevance and functional significance of cytokines to the adverse symptoms experienced by BC survivors from the earliest stages of their illness.

The positive association observed between Fatigue Severity and three of the cytokines (IL-6, IL-10, and TNF-α) pre-chemotherapy is consistent with expectations given that these cytokines are pro-

inflammatory (Haapakoski et al., 2015; Singer and Ouburg, 2016; Vos et al., 2016; Zhang et al., 2017), and fatigue was hypothesized in the current study to be at least in part attributable to increased systemic inflammatory response (Srinivas et al., 2016; Huh et al., 2017; Herz et al., 2014; Combarros et al., 2009). IL-6 and TNF-α are well established cell signalling proteins involved in systemic inflammation (Leong and Karsan, 2000; Interleukin-6 Receptor Mendelian Randomisation Analysis et al., 2012), including acute immune response (Heinrich et al., 1990) (Singh et al., 2018; Vos et al., 2016; Zhang et al., 2017), and neuroinflammation (Erta et al., 2012; Klegeris et al., 2007; Srinivas et al., 2016). That elevated IL-10 also had a positive relationship with Fatigue Severity may be indicative of the response of this cytokine synthesis inhibitor to modulate the pro-inflammatory response. IL-2 which had a negative association with Fatigue Severity, is a cytokine that plays a key role regulating leukocytes and lymphocytes, and reduced levels of IL-2 are common in response to cancer (Gaffen and Liu, 2004; Liao et al., 2011). In fact, IL-2 is sometimes given as a drug (Aldeslukein) to treat certain types of cancer (Noble and Goa, 1997; Bhatia et al., 2009). Therefore, fatigue associated with low levels of IL-2 likely reflects fatigue in response to cancer itself. The inverse relationship of MCP-1 and IL-12 with Fatigue Severity may also reflect the impact of having cancer on fatigue resulting from dysregulation of the immune system in the context of cancer.

Following the introduction of chemotherapy, significant increases in Fatigue Severity, Fatigue Interference, and Worst Fatigue occurred. Overall Fatigue Severity among the BC survivors increased from the mild range to moderate severity at the onset of chemotherapy. During that period, approximately half of the BC survivors reported moderate to severe fatigue. These results have several implications: 1) Fatigue was not a problem for all BC survivors, but was reported in approxmately two thirds of cases at baseline; 2) While mild Fatigue Severity was reported by the group on average, among BC survivors who reported fatigue, its severity was moderate and many reported severe fatigue during chemotherapy; 3) The Worst Fatigue level over the past 24-hours reported at the beginning of chemotherapy was greatest; 4) Many BC survivors also reported that their fatigue interfered with daily activities, and when only those women who reported any fatigue are considered, approximately half indicated a moderate to severe adverse impact; and 5) These findings contrasts with overall cognitive performance which remained in average to above average range for the group as whole across the study period. In sum, fatigue was commonly reported and increased dramatically during chemotherapy such that a substantial proportion of treated women reported moderate to severe fatigue and complained that fatigue was having a moderte to severe adverse impact, interfering with their daily activities. Accordingly, the results indicate that fatigue was a clinically signficant problem for many BC survivors prior to, during, and following chemotherapy. BC survivors' subjective fatigue symptoms were greater than overall cognitive deficits on objective neurocognitive tests.

The cytokines associated with fatigue changed during chemotherapy. Greater Fatigue Severity was associated with greater concentrations of IL-7 and TNF-α, but with reduced levels of IL-12. The cytokines IL-6 and IFN- γ were no longer significantly associated with Fatigue Severity during chemotherapy. Fatigue severity increased from baseline levels during chemotherapy, so the increase in fatigue was not linked to these two cytokines that are typically observed to be associated with systemic inflammation. IL-7 on the other hand is a cytokine that stimulates differentiation of pluripotent hematopoietic stem cells, has been found to be increase with certain malignancies, but also is important for immune system recovery and chemotherapy response, which may explain the relationship with Fatigue Severity. Interference with daily activities attributed to fatigue during chemotherapy was also associated with three of the same cytokines IL-7, IL-12 and TNF-α, but also IL-5. Like IL-7, elevated IL-5 occurred in women with less reported Fatigue Interference (Or et al., 1998; Rosenberg et al., 2006).

The relationship between cytokine concentrations and fatigue symptoms again changed following the completion of chemotherapy. Greater concentrations of IL-6 were associated with greater fatigue symptoms, whereas greater concentrations of IL-12 and MCP-1 were inversely associated with fatigue. Therefore, immediately after the completion of chemotherapy, the relationship between cytokine concentrations and fatigue became more similar to what had been observed prior to chemotherapy. Further change was observed by the time of the follow-up assessment. At 12-months post-chemotherapy as only IL-17 was associated with Fatigue Severity, with greater IL-17 concentrations occurring when there was less severe fatigue. The cytokines associated with Fatigue Interference at the 12-month also had changed, with elevated IL-12 corresponding to greater Fatigue Interference, but elevated IL-10 with less Fatigue Interference. By the 24-month follow-up assessment, the relationship between cytokine concentrations and fatigue appeared to normalize to pre-chemotherapy levels. At that 24-month follow-up, fatigue symptoms were most strongly associated with elevated IL-6, TNF-α and reduced IL-1β concentrations. The return of the fatigue-cytokine relationship to a pre-chemotherapy pattern is consistent with changes in reported fatigue symptoms over time.

The examination of the clinical variables in conjunction with the cytokines did not alter the overall relationships between cytokine concentrations and fatigue, but instead generally increased the amount of variance in fatigue that was accounted for. Only a single clinical covariate was associated with fatigue at most study time points, suggesting that the cytokines were contributing substantially to the symptoms of fatigue. Prior to and during chemotherapy, receptor positivity to progesterone or HER tended to be associated with more severe symptoms of fatigue. Typically, more aggressive forms of cancer occur when there is receptor positivity, which may account for more severe fatigue among these BC survivors, though notably tumor grade was not among the clinical factors associated with fatigue at any time point. By 12- and 24- months post-chemotherapy, treatment with neoadjuvant therapy was the clinical factor most strongly associated with fatigue. Neoadjuvant therapies are given prior to surgery to shrink the size of tumors to facilitate their removal. Their use implies a larger tumor which may also be indicative of a more aggressive or advanced cancer. Why this clinical factor was associated with fatigue at 12- and 24-month post-chemotherapy is not clear, but may reflect the impact of greater disease severity.

When cognitive function was examined simultaneously with the cytokines both tended to be associated with reported fatigue severity and functional interference, though again this varied based on the time point in the study. As expected, cognitive performance was inversely related to Fatigue Severity and interference; greater fatigue associated with weaker cognitive functioning. For the most part, the cytokines exhibited a similar relationship to fatigue as in the initial analyses when cognitive performance was not considered in the models, suggesting that the addition of cognitive performance in conjunction with the cytokines better accounted for fatigue. However, at certain study time points, either cognition or the cytokines were eliminated from the model indicating that the relative influence of the cytokines and cognition on fatigue varied based on whether or not active chemotherapy was occurring. Interestingly, when including all variables in the regression model, clinical variables such as menopause status, progesterone receptor positivity, and neoadjuvant therapy were retained as significantly related to fatigue at various time points, in addition to cognitive functioning performance. That neoadjuvant therapy remained significantly positively associated with the interference fatigue has on daily function at the 24-month follow-up is particularly interesting, and suggests that this type of therapy may contribute to long-term Fatigue Interference symptoms in BC survivors. However, since neoadjuvant therapy is also used to reduce tumor size prior to surgery, it is also possible that its association with fatigue indices long after the period of chemotherapy is a function of cancer severity in the BC survivors.

Prior to chemotherapy, both GCI and three cytokines (IL-2, IL-6, and IL-10) were associated with Fatigue Severity, whereas only GCI was

associated with the Fatigue Interference. This suggests that these cytokines and cognitive function may be affected by the cancer process. However, during and immediately following chemotherapy, cognition was not significantly associated with Fatigue Severity or Fatigue Interference despite continued relationships of IL-6, IL-7, and IL-12, which persisted through the end of chemotherapy (T3). That cognition was not associated with Fatigue Severity or Interference during or immediately following chemotherapy suggests the possibility that the neurotoxic effects of chemotherapy overrode the adverse impact of cognitive deficits on fatigue. By 12-months post-chemotherapy, the relationship between cognition, the cytokines and fatigue had changed, and cognition was again a significant factor accounting for fatigue symptoms. Twelve months following chemotherapy, IL-17 along with cognitive performance was associated with Fatigue Severity, whereas only cognitive performance was associated with Fatigue Interference. By 24-months post-chemotherapy, Both Fatigue Severity and Interference were reduced in comparison to during chemotherapy, returning to levels only that were only mildly elevated and Fatigue Interference to pre-chemotherapy levels. At that 24-month follow-up visit, IL-1β, IL-4, IL-6, IL-12, G-CSF, and TNF-α, along with global cognitive functioning were associated with Fatigue Severity. The relationship with G-CSF is potentially significant since chemotherapy tends to inhibit stem cell production by the bone marrow, so the association between G-CSF and Fatigue Severity at this time point may reflect the impact of recovery of this process.

Cognitive deficits involving attention, executive control, working memory and cognitive slowing are most strongly associated with Fatigue Severity and Fatigue Interference among BC survivors. The relationship between performance in these cognitive domains and fatigue was evident in the current study, though they had different longitudinal trajectories. That serum cytokine concentrations, but not cognitive performance, were associated with the adverse functional consequences of fatigue during the chemotherapy period reinforces the conclusion that fatigue likely plays a major role in the adverse symptoms attributed to chemotherapy. The fact that cognitive deficits were associated with greater fatigue, also suggests that such deficits likely make cognitive processing more effortful. Therefore, the fatigue experienced by BC survivors may in part reflect cognitive fatigue. Yet, the fact that cytokine concentrations were also associated with fatigue independent of cognitive performance, particularly during the period of chemotherapy when fatigue was greatest, suggests that alterations in immune system and inflammatory response likely underlie the exacerbation of these symptoms. On a positive note, both fatigue symptoms and cognitive performance improved over the two-year period of the study following the completion of chemotherapy. Therefore, chemotherapy did not cause either persistent fatigue or cognitive dysfunction in the major of BC survivors and it is unlikely that permanent chemotherapy-associated structural or functional brain abnormalities occurred.

4.1. Study limitations

Conclusions that can be reached from this study are subject to certain limitations, including: (a) lack of age-and gender-matches BCSs not receiving cancer treatment (i.e. comparative controls); (b) absence of pre-cancer diagnosis/surgery cognitive function assessment; and (c) increased familiarity with the assessment measures through repeated administration. Because most patients undergo multiple treatment modalities upon the diagnosis of breast cancer, the inclusion of a control group was not feasible in the current study as it would have been difficult to discern correlations among specific cancer treatment modalities with cognitive status and fatigue findings. Future studies involving both age- and gender-matched BCSs not receiving chemotherapy as well as control group of non-cancer patients would strengthen the ability to statistically adjust for the multitude of factors that could influence the effects of cancer treatment on outcome variables. Additionally, stratifying study participants according to specific type of cancer treatment administered would contribute to a strengthened study design, though this would require a much larger sample size. Future studies employing larger sample sizes would enable more precise and reliable statistical models adjusting for covariates are needed to estimate the differences in expected versus actual fatigue at baseline. (Lyon et al., 2016a; Lyon et al., 2016b; Andreotti et al., 2016).

In the current study, fatigue was assessed at first assessment following BC diagnosis, but prior to chemotherapy. It seems likely that fatigue at this time was caused by other adverse physiological effects of the cancer, or comorbid factors existing at the time of diagnosis. However, data regarding the fatigue experienced by participants prior to their cancer diagnosis was not available, so the prevalence of premorbid fatigue symptoms can be determined. The challenge of obtaining pre-cancer diagnosis/surgery cognitive status seems insurmountable as routine healthcare visits generally do not include such data collection. As in past studies, fatigue was assessed via ratings, providing measures of the BC survivors' subjective experience of fatigue. While these subjective experiences are important in the context of clinical care and also the quality of life of BC survivors, they may not be indicative of objective behavioral or physiological fatigue occurring during task performance or everyday activities.

Whether the subjective experience of fatigue is the primary basis for symptoms of chemobrain as reported by many BC survivors is not entirely clear. It seems likely that this may be the case given that fatigue symptoms were more common and had greater reported severity than did actual cognitive deficits. “Chemobrain” is ultimately a colloquial term is widely gained widespread use by the lay public for describing self-perceived alterations in mental status attributed to chemotherapy effects. In this study, we did not specifically examine whether BC survivors felt that they were experiencing chemobrain or whether they would describe their subjective fatigue symptoms as chemobrain.

4.2. Conclusions

The findings of this study demonstrate that systemic cytokine concentrations are associated with the severity and functional impact of fatigue experienced by BC survivors. This relationship is complex and varies based on study phase. Prior to and at long-term follow-up, 12- and 24-months post-chemotherapy, there is relative consistency in the cytokines that are associated with fatigue, including several of interleukins and TNF-α. At these time points, cognitive function is also associated with fatigue symptoms but not during or immediately after the completion of chemotherapy when examined simultaneously with the cytokines. During chemotherapy, fatigue symptoms increase and the cytokines have a stronger relationship to fatigue than does cognition, which exerts less influence.

The clinical implication is that fatigue is a major manifestation of chemotherapy that may be driven by the response to pro-inflammatory cytokines, and together these relationships likely account for much of the adverse symptomatology experienced by BC survivors (i.e., “chemobrain”). Fatigue during the chemotherapy period is likely exacerbated by the neurotoxic effects of the intervention, with cytokine alterations a significant contributing factor. Fortunately, fatigue symptoms tend to lessen over two years following chemotherapy. Cognitive deficits also exist in many BC survivors, but are present prior to chemotherapy, show only small increases during chemotherapy, and return to pre-chemotherapy levels over the long-term (Lyon et al., 2016b). Prior to and following chemotherapy, BC survivors with cognitive deficits report greater fatigue, which may be a manifestation of cognitive fatigue linked to greater effort required to complete tasks.

Ultimately, the temporal relationship between chemotherapy, fatigue and cognition, provides insights into how cytokine concentrations along with cognitive status affect fatigue from pre-chemotherapy through 2-year survivorship. This information may be useful to health care providers dealing with symptom management for BC survivors

undergoing different phases of the study; as part of patient education, provider-patient discussions for preventive strategies, and symptom management, and efforts to improve quality of life. To optimize treatment consequences and daily activities before, during, and after chemotherapy, we suggest that patients would benefit from interventions targeted towards fatigue, with an emphasis on tailoring interventions based on cognitive status and psychosocial comorbidities. During chemotherapy management of drug side effects and cytokine alterations may be more important, though this still should be addressed in the context of these other factors.

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Conflict of interest statement

Authors, Cohen, Gullett, and Lyon declare that they have no conflict of interest.

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