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Fatigue and herpesvirus latency in women newly diagnosed with breast cancer

Christopher P. Fagundes [a,*], Ronald Glaser [a,b,c,d], Catherine M. Alfano [e], Jeanette M. Bennett [a,f], Stephen P. Povoski [d,g], Adele M. Lipari [h], Doreen M. Agnese [d,g], Lisa D. Yee [d,g], William E. Carson III [b,d,g], William B. Farrar [d,g], William B. Malarkey [a,b,c,d,i], Janice K. Kiecolt-Glaser [a,d,i]

[a] Institute for Behavioral Medicine Research, The Ohio State University College of Medicine, USA
[b] Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University College of Medicine, USA
[c] Department of Internal Medicine, The Ohio State University College of Medicine, USA
[d] Comprehensive Cancer Center, The Ohio State University College of Medicine, USA
[e] Office of Cancer Survivorship, National Cancer Institute, National Institute of Health, USA
[f] Division of Oral Biology, The Ohio State University College of Dentistry, USA
[g] Department of Surgery, The Ohio State University College of Medicine, USA
[h] Department of Radiology, The Ohio State University College of Medicine, USA
[i] Department of Psychiatry, The Ohio State University College of Medicine, USA

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ABSTRACT

Fatigue is a notable clinical problem in cancer survivors, and understanding its pathophysiology is important. The current study sought to determine biomarkers of fatigue that exist before cancer treatment. Relationships between the expression of latent Epstein–Barr virus (EBV) and cytomegalovirus (CMV) and fatigue were examined in 158 women newly diagnosed with breast cancer or awaiting a positive diagnostic result. Higher CMV antibody titers, but not EBV antibody titers, were associated with a greater likelihood of being fatigued. Associations between fatigue and higher CMV antibody titers remained after controlling for alcohol use, smoking, comorbidities, depressive symptoms, age, BMI, cancer stage, and sleep problems. More sleep problems and higher levels of depressive symptoms were also associated with a greater likelihood of being fatigued. CMV antibody titers, but not EBV antibody titers, were associated with higher levels of C-reactive protein (CRP), but CRP was not associated with fatigue. When the cellular immune system is compromised, reactivation of latent herpesviruses may fuel chronic inflammatory responses. Prior work has suggested that fatigue may be related to inflammation and its associated sickness behaviors; accordingly, our findings may be tapping into this same physiological substrate.

1. Introduction

Fatigue is a common problem among those treated for breast cancer, as well as long-term breast cancer survivors (Bower et al., 2006; Butt et al., 2008; Cleeland et al., 2003; Ganz et al., 2002; Jacobsen et al., 2007). Fatigue adversely affects overall quality of life, as well as many daily activities including mood, the sleep–wake cycle, and personal relationships (Bower et al., 2002; Collado-Hidalgo et al., 2006; Lawrence et al., 2004). Understanding the factors that contribute to fatigue is important. The literature to date has focused on cancer fatigue during and after treatment (Bower, 2007; Jacobsen et al., 1999). We sought to examine biomarkers of pretreatment fatigue that may be exacerbated by cancer therapies.

Bower and her colleagues have demonstrated that cancer survivors who report persistent fatigue are characterized by higher levels of inflammation after treatment than non-fatigued cancer survivors (Bower, 2007). For example, fatigued breast cancer survivors had higher levels of proinflammatory activity including interleukin-1 receptor antagonist (IL-1ra), soluble tumor necrosis factor receptor Type II (sTNF-RII), and neopterin than breast cancer survivors who were not fatigued (Bower et al., 2002). In another study, fatigued survivors had higher levels of soluble IL-1ra and soluble interleukin-6 receptor (sIL-6r) than non-fatigued survivors (Bower et al., 2003). Similarly, *ex vivo* production of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) by lipopolysaccharide (LPS) stimulated monocytes was higher among fatigued compared to non-fatigued breast cancer survivors (Collado-Hidalgo et al., 2006).

Maladaptive pretreatment alterations in immune function that promote inflammation could promote subsequent cancer-related fatigue. Recent research has highlighted links between herpesvirus reactivation and inflammation. Herpesviruses create persistent

* Corresponding author. Address: Institute for Behavioral Medicine Research, Ohio State University College of Medicine, 460 Medical Center Drive, Columbus, OH 43210, USA.
E-mail address: christopher.fagundes@osumc.edu (C.P. Fagundes).

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latent infections; once a person has been infected with one of the herpesviruses, they will carry the virus for the rest of their life (Glaser and Kiecolt-Glaser, 1994). Herpesviruses remain dormant in latently infected cells. Elevated antibody titers to a latent herpesvirus reflect poorer cellular immune system control over viral latency (Glaser and Kiecolt-Glaser, 1994). When the cellular immune system is compromised, the virus may be triggered to reactivate and replicate in latently infected cells; these interactions result in chronic inflammatory responses (Glaser and Kiecolt-Glaser, 2005; Glaser et al., 2005a,b; Steptoe et al., 2007).

Elevated cytomegalovirus (CMV) antibody titers have been associated with increased IL-6 and TNF-α production (Roberts et al., 2010). Furthermore, viral proteins synthesized during Epstein–Barr virus (EBV) replication can enhance production of the proinflammatory cytokines IL-6, TNF-α, and interleukin-1 beta (IL-1β) (Glaser et al., 2006). How effectively latent herpesviruses are being controlled by the cellular immune response before treatment could have important implications for fatigue.

We examined relationships between fatigue and EBV and CMV reactivation in a sample of newly diagnosed breast cancer patients who had not yet received cancer treatment. We sought to identify pretreatment biomarkers of fatigue. We also examined relationships between viral reactivation and C-reactive protein (CRP), an acute phase protein that is a downstream marker of inflammation. CRP was the only inflammatory marker available, and other inflammatory markers likely also play an important role. We hypothesized that higher antibody titers to EBV and CMV would be associated with a greater risk for fatigue. We also hypothesized that higher antibody titers to EBV and CMV would be associated with higher levels of CRP. As an ancillary analysis, we examined the possibility that CRP partially explained relationships between antibody titers to EBV and CMV and fatigue.

2. Methods

2.1. Subjects

Participants were recruited for a larger study on cancer-related fatigue and immune function. They were referred to us by participating physicians or their nurse practitioners, and some were recruited when medical record review indicated eligibility. Screening exclusions included a prior history of breast or any other cancer except basal or squamous cell skin cancers. Of the 254 women who were approached, 167 chose to participate. They completed a questionnaire battery and blood draw prior to their cancer treatment, including surgery. One hundred and fifty-eight women (95%) were EBV seropositive and 97 (58%) were also seropositive for CMV, consistent with epidemiological data (Staras et al., 2006; WHO, 2008). The vast majority of women knew their positive diagnosis at the time of the blood draw (90%). All participants who were seropositive for CMV were seropositive for EBV. Women did not differ on any of the study variables depending on their seropositive status or diagnosis knowledge. The Institutional Review Board approved the project; all subjects gave written informed consent prior to participation.

2.2. Determination of EBV VCA and CMV IgG antibody titers in plasma

Plasma was stored at −80 °C until assayed with Euroimmun EBV ELISA plates that measure EBV virus capsid antigen (VCA) antibody titers (Morris Plains, NJ). CMV IgG antibody titers were also determined using Euroimmun CMV ELISA plates (Morris Plains, NJ). CMV and EBV VCA IgG antibody titers were assessed following company instructions with some modifications. Specifically, for each ELISA plate three controls that were included in each kit (one positive sample, one negative sample, and three calibrators) were run in duplicate. Plasma samples were initially diluted 1:101 with a dilution buffer according to the recommended protocol provided by the company. Then, six serial two-fold dilutions of each sample were assayed. The last dilution factor with a positive IgG value determined the IgG antibody titer. Calculated viral titers for each sample were plotted and samples were rerun if the end point did not fall within the linear range (±15%). CMV IgG antibody titers were determined following the same protocol as EBV VCA IgG antibody titers, except the samples were initially screened for seropositive status. Only CMV seropositive samples were serially diluted to assess the CMV antibody titer. Antibody titers were treated as continuous variables in all of our analyses based on the extant literature showing that latent virus reactivation occurs to varying degrees, and therefore should be represented as continuous (Glaser and Jones, 1994).

2.3. CRP

The high-sensitivity C-reactive protein (hsCRP) assay was performed, using chemiluminescence methodology with Immulite 1000 (Siemens Medical Solutions, Los Angeles, California). The lowest level of detection is 0.3 mg/L. Intra-assay coefficient of variation is 5.1% and interassay coefficient variation is 7.3%.

2.4. Measures

The RAND SF-36 vigor/vitality scale (Ware et al., 1992) was used because of its use in a series of studies assessing the biological mechanisms underlying cancer-related fatigue (Bower, 2007; 2005a; 2006). Standardized scores on the RAND SF-36 vigor/vitality scale range from 0-100, with higher scores indicating less fatigue (Ware et al., 1992). A score of greater than 50 represents well-being. A "case categorical" variable has been commonly used in studies assessing the biological mechanisms underlying cancer-related fatigue (Bower, 2007; Bower et al., 2000; 2002; 2003; 2005a,b; 2007; 2006; 2009; Fagundes et al., 2011). In accord with prior work, those who scored above 50 were considered non-fatigued, while those who scored 50 or below were considered fatigued (Bower et al., 2000; Fagundes et al., 2011).

The Center for Epidemiological Studies Depression Scale (CES-D) has been used extensively as a brief measure of depressive symptomatology (Basco et al., 1997; Radloff, 1977). Studies have shown acceptable test–retest reliability and excellent construct validity (Basco et al., 1997). As the CES-D has also distinguished depressed from non-depressed participants in community and clinical samples, discriminative validity appears acceptable as well (Basco et al., 1997). It has been widely used in cancer studies (Demark-Wahnefried et al., 2003). In this sample, Cronbach's alpha was .92.

We used the Charlson Index to obtain information about comorbidities. The Charlson Index is the most widely used comorbidity index (Charlson et al., 1994). Widely used with both cancer and noncancer populations (Dobnig et al., 2008), the measure assesses the presence of connective tissue diseases, diabetes with/without end organ damage, chronic pulmonary disease, ulcers, peripheral vascular disease, cerebrovascular disease, moderate to severe renal disease, other cancers, metastatic cancer, liver disease, myocardial infarction, congestive heart failure, hemiplegia, dementia, leukemia, lymphoma, moderate to severe liver disease, and acquired immune deficiency syndrome. It assigns weights to these conditions based on their potential influence on one-year mortality in breast cancer patients. We obtained comorbid information from both medical charts and subjects' self-reports.

The Insomnia Severity Index (Morin, 1993) has been used to collect reliable data on insomnia severity (Bastien et al., 2001), including data from cancer patients (Savard et al., 2005). Higher

numbers indicate greater insomnia severity. In this sample, Cronbach's alpha was .89.

Participants answered questions about their age, race, marital status, smoking status, and weekly average alcohol consumption. Breast cancer stage data were obtained through the Cancer Registry and electronic medical records.

2.5. Analytic method

Both antibody titers and CRP data were log transformed to normalize their distributions prior to analyses. Multiple logistic regression analyses assessed relationships between EBV and CMV antibody titers and fatigue status. An initial regression analysis was conducted with the 158 women who were EBV seropositive examining the relationship between EBV and fatigue; the same analysis was performed once again in a subset of the EBV seropositive women who were also CMV seropositive examining relationships between EBV, CMV, and fatigue ($N = 97$). All CMV seropositive women were also EBV seropositive. Adjustments were made for age, depressive symptoms, BMI, sleep, alcohol consumption, comorbidities, smoking status, and cancer stage.

Ordinary least squares multiple linear regression analysis assessed relationships between EBV antibody titers, CMV antibody titers, and CRP levels. An initial regression analysis assessed relationships between EBV and CRP in the women who were EBV seropositive. A subsequent analysis was conducted for the women who were both EBV and CMV seropositive assessing relationships between EBV, CMV, and CRP. Adjustments were made for age, BMI, sleep, alcohol consumption, comorbidities, smoking status, cancer stage, and medications that may impact inflammation. If antibody titers predicted fatigue status and CRP levels, we planned to examine whether CRP mediated the relationship between antibody titers and fatigue.

3. Results

Table 1 presents sample population characteristics between fatigued and non-fatigued women, as well as unadjusted differences between fatigued and non-fatigued women. EBV and CMV were moderately correlated ($r = .28$, $p < .01$). Seropositive status to CMV and/or EBV was unrelated to fatigue. EBV and CMV antibody titers for fatigued and nonfatigued breast cancer patients are graphically illustrated in Figs. 1 and 2, respectively.

Table 2 summarizes analyses that addressed relationships between EBV antibody titers and fatigue in the women who were EBV seropositive and in the subset of women who were both EBV and CMV seropositive. EBV antibody titers did not emerge as a significant predictor of fatigue status. However, greater alcohol use (trend level), higher levels of depressive symptoms, and poorer sleep were all associated with a greater likelihood of being fatigued. In the regression analyses with women who were both EBV and CMV seropositive, EBV antibody titers once again did not predict fatigue status. However, those who had higher CMV antibody titers were more likely to be fatigued than those who had lower CMV antibody titers. Poorer sleep quality and higher levels of depressive symptoms were once again associated with a greater likelihood of being fatigued. EBV and CMV antibody titers did not interact to predict fatigue status, so the interaction term was dropped from the model. The association between CMV antibody titers and fatigue remained in an additional analysis that excluded sleep quality and depressive symptoms from the model. In ancillary analyses, fatigue was modeled as a continuous dependent variable using ordinary least squares multiple regression; higher levels of CMV antibody titers were associated with greater fatigue ($p < .04$). Furthermore, the significance levels of all other variables remained the same.

Table 3 summarizes the analyses that addressed relationships between EBV antibody titers and CRP in the group of women who were EBV seropositive and the group of women who were both EBV and CMV seropositive. Among women who were only EBV seropositive, EBV antibody titers did not emerge as a significant predictor of CRP. However, having a higher BMI and a later cancer stage was associated with having higher CRP levels. In the subset of women who were both EBV and CMV seropositive, EBV antibody titers once again did not predict CRP levels. However, those who had higher levels of CMV antibody titers had higher levels of CRP. Those who smoked had higher levels of CRP than those who did not smoke. Furthermore having a higher BMI once again was associated with having higher levels of CRP.

Given that higher CMV antibody titers were associated with a greater likelihood of being fatigued, as well as higher CRP levels, we explored the possibility that CRP mediated the association between CMV antibody titers and fatigue. We added CRP to the second regression model presented in Table 2. CRP was not associated with the odds of being fatigued. We then generated 95% bias-correct bootstrap confidence intervals (CI) for the indirect effect of CRP using 5000 bootstrap samples (MacKinnon et al., 2002; MacKinnon et al., 2004). There was not a significant indirect effect (95% CI: −.88–1.43); therefore, CRP did not mediate the relationship between CMV antibody titers and fatigue.

4. Discussion

Fatigue is a notable clinical problem, and thus a better understanding of the factors that contribute to its development is important. Among women newly diagnosed with breast cancer, higher CMV antibody titers, but not EBV antibody titers, were associated with a greater likelihood of being fatigued. Likewise, CMV antibody titers, but not EBV antibody titers, were related to higher CRP. CRP was not associated with fatigue. More sleep problems and higher levels of depressive symptoms were also associated with a greater likelihood of being fatigued.

EBV and CMV reactivation are influenced by different mechanisms. Accordingly, it may not be surprising that CMV antibody titers were linked to fatigue in our study, while EBV antibody titers were not. For example, astronauts had different patterns of latent reactivation of EBV and CMV during space flight (Mehta and Pierson, 2007). Similarly, latent CMV and EBV reactivated differently during academic stress (Matalka et al., 2000). Indeed, *in vivo* reactivation of different herpesviruses may involve multiple neuroendocirine interactions (Glaser et al., 1985; Kiecolt-Glaser et al., 1984; Kiecolt-Glaser and Glaser, 1987; Yang et al., 2010). Further work is necessary to understand these complex processes (Matalka et al., 2000; Mehta and Pierson, 2007; Steptoe et al., 2009).

There is considerable evidence linking CMV to inflammation, which may explain the relationship between CMV antibody titers and fatigue. For example, *in vitro* studies suggest that CMV promotes proinflammatory cytokine production in human cultured cells (Almeida et al., 1994). In both healthy and patient populations, IL-6 and CRP have been associated with higher CMV antibody titers (Blankenberg et al., 2001; Nazmi et al., 2010). Higher CMV antibody titers were also associated with all-cause mortality, and this relationship was largely explained by elevated IL-6 and TNF-α (Roberts et al., 2010). There is also work to suggest that CMV, and to a lesser extent EBV, contributes to immunosenescence (Pawelec et al., 2009), although it has been much less studied. EBV encoded deoxyuridine triphosphate nucleotidohydrolase (dUTPase), a protein synthesized in the early phase of virus replication, can induce human monocytes/macrophages to produce proinflammatory cytokines (Glaser et al., 2006).

Table 1
Sample population characteristics.

Variable	Total (N = 158)			Fatigued (n = 72)			Non-fatigued (n = 86)			Test statistic[c]	p
	n	M	SD	n	M	SD	n	M	SD		
EBV		1839.73	1685.02		1851.67	1777.16		1829.74	1614.32		
CMV		1651.40	1489.68		1805.10	1408.60		1506.92	1562.40		
CRP		3.19	5.29		3.13	4.48		3.24	5.89		
EBV ($\log_{10}$)		3.09	.40		3.08	.44		3.11	.37	.31	.58
CMV ($\log_{10}$)[a]		3.06	.41		3.16	.30		2.96	.47	5.78	.02
CRP ($\log_{10}$)[b]		.14	.54		.15	.53		.14	.55	.02	.88
Age (yrs)		55.20	11.80		53.40	11.44		56.71	11.95	3.12	.08
Race											
White	127			61			66			3.48	.48
Black	24			9			15				
Asian	4			2			2				
Native American	1			0			1				
Other	2			0			2				
Sleep		8.44	5.81		11.38	5.84		5.99	4.52	42.64	.00
Alcohol (drinks/week)		1.87	3.77		1.61	4.49		2.09	3.06	.64	.43
Smoker	23			12			11			.47	.49
Breast Cancer Stage										.15	.70
0	26			14			12				
I	57			27			30				
IIA	31			11			20				
IIB	20			10			10				
IIIA	11			4			7				
IIIB	1			0			1				
IIIC	4			1			3				
IV	8			5			3				
Depressive Symptoms		15.28	11.05		21.03	10.45		10.46	9.10	46.12	.00
BMI		28.61	7.02		28.76	5.82		28.48	7.91	.06	.81
Comorbidities		.63	1.19		.63	1.07		.64	1.3	.01	.93
Rand SF-36 Vigor/Vitality		54.37	21.88		34.44	13.15		71.05	11.04		

[a] 97 women were CMV seropositive (47 fatigued, 50 non-fatigued).
[b] 132 women had CRP data available (59 fatigued, 73 non-fatigued).
[c] All test statistics represent F-values other than those that correspond to smoker and race, which represent chi-square values.

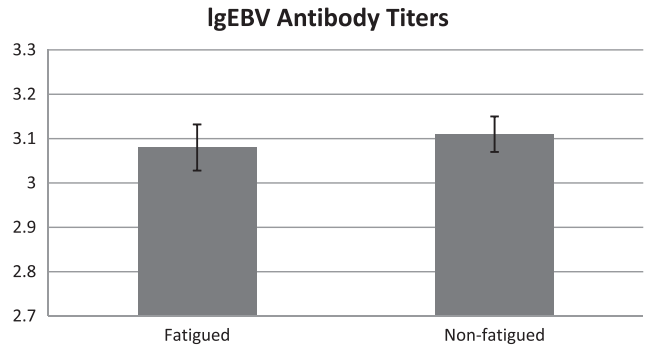

Fig. 1. Epstein–Barr virus (EBV) antibody titers (log-transformed) for fatigued and nonfatigued breast cancer patients.

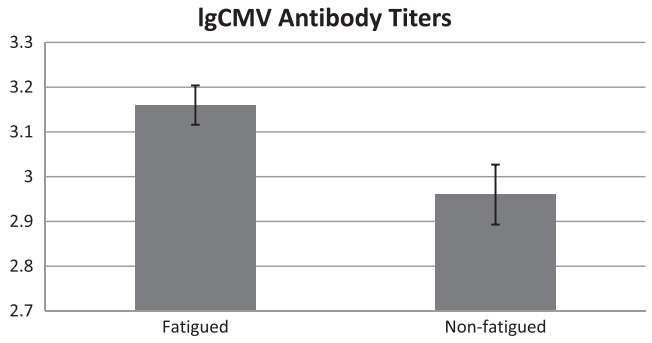

Fig. 2. Cytomegalovirus (CMV) antibody titers for fatigued and nonfatigued breast cancer patients.

CRP was associated with higher CMV antibody titers; however, it was not directly linked to fatigue. CRP is produced by hepatocytes in response to proinflammatory cytokines (Libby et al., 2002). It is synthesized by the liver and highly sensitive to other factors unrelated to proinflammatory cytokines (Koenig, 2003; Libby et al., 2002). Accordingly, it is not surprising that CRP was unrelated to fatigue even though there is considerable evidence linking proinflammatory cytokines to fatigue.

Although we are unaware of any studies that have simultaneously assessed inflammation and fatigue before and after treatment to date, one study demonstrated that the number of fatigued breast cancer survivors remain constant before and 1, 2, and 3 years post-treatment (Nieboer et al., 2005). Furthermore, fatigue remained stable among a proportion of these women across time points (Nieboer et al., 2005). Based on evidence linking inflammation and fatigue among long-term breast cancer survivors, pretreatment differences in the steady state expression of latent herpesviruses could be an important biomarker for persistent fatigue.

During primary infection, EBV and CMV can cause mononucleosis, in which fatigue is a prominent symptom (Klemola et al., 1970). In one study, exhausted individuals were more likely to be seropositive for herpesviruses varicella-zoster virus (VZV) and CMV than controls (Van Der Ven et al., 2003). Furthermore, herpesvirus reactivation has been linked to chronic fatigue syndrome (Glaser et al., 2005a,b). However, to our knowledge, there is no work that has linked either CMV or EBV antibody titers to fatigue in otherwise healthy adults. This is an important direction for future work.

Table 2
Adjusted logistic regression analysis predicting fatigued status.

Model	Variable	*B*	Odds ratio	95% CI for Odds Ratio	*p*-Value
Women EBV Seropositive	EBV (log_{10})	−.36	.70	.26–1.90	.48
	Alcohol (drinks/week)	−.09	.91	.83–1.01	.07
	Smoker (1 = current)	−.27	.76	.24–2.41	.65
	Comorbidities	−.02	.98	.70–1.37	.91
	Depressive Symptoms	.08	1.08	1.04–1.14	.00
	Age (yrs)	.00	.10	.96–1.03	.82
	BMI (kg/m^2)	.00	.10	.94–1.05	.88
	Cancer stage	−.06	.94	.74–1.19	.61
	Sleep	.14	1.16	1.06–1.26	.00
	χ^2 (9, N = 158) = 55.95				.00
	R^2(Negelkerke) = .40				
Women both EBV and CMV Seropositive	EBV (log_{10})	−.61	.54	.13–2.31	.41
	CMV (log_{10})	1.95	7.01	1.45–33.95	.02
	Alcohol (drinks/week)	−.09	.91	.81–1.03	.12
	Smoker (1 = current)	−.55	.58	.11–3.06	.52
	Comorbidities	.07	1.07	.66–1.73	.78
	Depressive Symptoms	.08	1.09	1.02–1.16	.01
	Age (yrs)	.00	.10	.95–1.04	.91
	BMI (kg/m^2)	−.06	.94	.87–1.02	.12
	Cancer stage	−.03	.97	.71–1.32	.84
	Sleep	.15	1.16	1.04–1.31	.01
	χ^2 (10, N = 97) = 42.16				.00
	R^2(Negelkerke) = .47				

Table 3
Adjusted ordinary least squares regression analyses predicting CRP.

Model	Variable	*B*	95% CI	*p*-value
Women EBV seropositive	EBV (log_{10})	.01	−.21–.22	.95
	Alcohol (drinks/week)	.00	−.02–.03	.76
	Smoker (1 = current)	.21	−.05–.46	.11
	Comorbidities	−.03	−.10–.05	.53
	Age (yrs)	.01	−.00–.01	.20
	BMI (kg/m^2)	.03	.02–.04	.00
	Cancer stage	.06	.01–.11	.02
	Sleep	−.01	−.02–.01	.33
	Inflammation-related Meds	.04	−.14–.22	.66
	F(9, 122) = 4.96			.00
	R^2 = .27			
Women both EBV and CMV seropositive	EBV (log_{10})	−.13	−.40–.15	.37
	CMV (log_{10})	.34	.04–.63	.02
	Alcohol (drinks/week)	.00	−.02–.03	.76
	Smoker (1 = current)	.26	−.08–.59	.13
	Comorbidities	.03	−.07–.13	.57
	Age (yrs)	.01	−.00–.02	.07
	BMI (kg/m^2)	.03	.02–.05	.00
	Cancer stage	.05	−.01–.11	.09
	Sleep	−.01	−.02–.01	.41
	Inflammation-related Meds	−.04	−.27–.19	.72
	F(10, 67) = 5.42			.00
	R^2 = .45			

Chemotherapy promotes herpesvirus reactivation by dysregulating cellular immunity (Kuo et al., 2008). Among cancer patients who were assessed before chemotherapy and then followed through treatment, both CMV IgG antibody titers and CMV viral load in leukocytes increased (Kuo et al., 2008). The proinflammatory cytokines TNF-α and interferon-gamma (IFN-γ) were also substantially higher following reactivation compared to before (Kuo et al., 2008).

Breast cancer patients are confronted with many problems during cancer treatment and its aftermath that can affect their well-being (Mols et al., 2005; Reich et al., 2008). Psychological stress and depression can promote herpesvirus reactivation or replication by impairing the ability of the cellular immune system to control viral latency (Glaser and Kiecolt-Glaser, 1994). For example, patients with coronary artery disease who were more depressed had higher CMV antibody titers than their less depressed counterparts. They also had higher levels of L-1β and TNF-α (Appels et al., 2000). It would be interesting to follow women with breast cancer before, during, and after treatment to investigate relationships among depression, immune dysregulation, and fatigue.

Although those who had higher levels of depressive symptoms were more likely to be fatigued, CMV antibody titers were associated with fatigue in our sample independent of depression. Our sample reported elevated depressive symptoms, even compared to other pretreatment cancer samples (Ancoli-Israel et al., 2006; Black and Markides, 1999; Liu et al., 2009; Polsky et al., 2005; Wu and Andersen, 2010). Depressive symptoms were not associated with either EBV or CMV antibody titers. However, a more

systemic assessment of stress using multiple measures might have allowed us to identify relationships between psychological stress and antibody titers.

CMV antibody titers may have implications beyond fatigue. Inflammation, which is enhanced during CMV reactivation, promotes mammary tumor development (DeNardo and Coussens, 2007). Furthermore, inflammation has been implicated in tumor promotion, survival, proliferation, invasion, angiogenesis, and metastases (Aggarwal et al., 2006; Coussens and Werb, 2002).

Most of the work on fatigue in women with breast cancer has been conducted post-treatment. Women were more fatigued in our sample compared with posttreatment samples of breast cancer survivors (Bower et al., 2000). This may have been due to variety of psychological and biological factors. The stress associated with a recent cancer diagnosis may promote fatigue; the disease may also play a role. The range of fatigued and non-fatigued women was a notable strength of our study.

We focused exclusively on women in our study. However, we do not know if our findings generalize to men or other types of cancer, a limitation of our study. Our sample was predominately white, a limitation of our study that could be addressed in future work. The women who refused to participate may have chosen to do so because they were more fatigued than their counterparts. However, we took precautions to prevent this by offering home visits to reduce participant demand. If the women who chose not to participate were indeed more fatigued, it is possible that our findings would be even stronger.

In sum, fatigue is an important health concern and understanding its pathophysiology is of great importance. Our data suggest that CMV may play an important role for fatigue among those newly diagnosed with cancer. Cancer treatment can further exacerbate the steady state expression of latent CMV by diminishing cellular immunity. CMV antibody titers could be an important objective biomarker of fatigue.

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References

Aggarwal, B.B., Shishodia, S., Sandur, S.K., Pandey, M.K., Sethi, G., 2006. Inflammation and cancer: How hot is the link? Biochemical Pharmacology 72 (11), 1605–1621.

Almeida, G. D., Porada, C., St Jeor, S., Ascensao, J. (1994). Human cytomegalovirus alters interleukin-6 production by endothelial cells. Blood, 83(2), 370.

Ancoli-Israel, S., Liu, L.Q., Marler, M., Parker, B.A., Jones, V., Sadler, G.R., et al., 2006. Fatigue, sleep, and circadian rhythms prior to chemotherapy for breast cancer. Supportive Care in Cancer 14 (3), 201–209.

Appels, A., Bär, F.W., Bär, J., Bruggeman, C., de Baets, M., 2000. Inflammation, depressive symptomatology, and coronary artery disease. Psychosomatic Medicine 62 (5), 601.

Basco, M.R., Krebaum, S.R., Rush, A.J., 1997. Outcome measures of depression. In: Strupp, H.H., Horowitz, L.M., Lambert, M.J. (Eds.), Measuring Patient Changes in Mood, Anxiety, and Personality Disorders. American Psychological Association, Washington DC, pp. 207–245.

Bastien, C.H., Vallieres, A., Morin, C.M., 2001. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. Sleep Medicine Reviews 2 (4), 297–307.

Black, S.A., Markides, K.S., 1999. Depressive symptoms and mortality in older Mexican Americans. Annals of Epidemiology 9 (1), 45–52.

Blankenberg, S., Rupprecht, H.J., Bickel, C., Espinola-Klein, C., Rippin, G., Hafner, G., et al., 2001. Cytomegalovirus infection with interleukin-6 response predicts cardiac mortality in patients with coronary artery disease. Circulation 103 (24), 2915.

Bower, J.E., 2007. Cancer-related fatigue: links with inflammation in cancer patients and survivors. Brain, Behavior, and Immunity 21, 863–871.

Bower, J.E., Ganz, P.A., Aziz, N., 2005a. Altered cortisol response to psychologic stress in breast cancer survivors with persistent fatigue. Psychosomatic Medicine 67, 277–280.

Bower, J.E., Ganz, P.A., Aziz, N., Fahey, J.L., 2002. Fatigue and proinflammatory cytokine activity in breast cancer survivors. Psychosomatic Medicine 64 (4), 604–611.

Bower, J.E., Ganz, P.A., Aziz, N., Fahey, J.L., Cole, S.W., 2003. T-cell homeostasis in breast cancer survivors with persistent fatigue. Journal of the National Cancer Institute 95 (15), 1165–1168.

Bower, J.E., Ganz, P.A., Aziz, N., Olmstead, R., Irwin, M.R., Cole, S., 2007. Inflammatory responses to psychological stress in fatigued breast cancer survivors: relationship to glucocorticoids. Brain, Behavior and Immunity 21, 251–258.

Bower, J.E., Ganz, P.A., Desmond, K.A., Bernaards, C., Rowland, J.H., Meyerowitz, B.E., et al., 2006. Fatigue in long-term breast carcinoma survivors: a longitudinal investigation. Cancer 106 (4), 751–758.

Bower, J.E., Ganz, P.A., Desmond, K.A., Rowland, J.H., Meyerowitz, B.E., Belin, T.R., 2000. Fatigue in breast cancer survivors: occurrence, correlates, and impact on quality of life. Journal of Clinical Oncology 18 (4), 743–753.

Bower, J.E., Ganz, P.A., Dickerson, S.S., Petersen, L., Aziz, N., Fahey, J.L., 2005b. Diurnal cortisol rhythm and fatigue in breast cancer survivors. Psychoneuroendocrinology 30, 92–100.

Bower, J.E., Ganz, P.A., Tao, M.L., Hu, W., Belin, T.R., Sepah, S., et al., 2009. Inflammatory biomarkers and fatigue during radiation therapy for breast and prostate cancer. Clinical Cancer Research 15 (17), 5534–5540.

Butt, Z., Rosenbloom, S., Abernethy, A., Beaumont, J., Paul, D., Hampton, D., et al., 2008. Fatigue is the most important symptom for advanced cancer patients who have had chemotherapy. Journal of the National Comprehensive Cancer Network 6 (5), 448.

Charlson, M., Szatrowski, T.P., Peterson, J., Gold, J., 1994. Validation of a combined comorbidity index. Journal of Clinical Epidemiology 47 (11), 1245–1251.

Cleeland, C.S., Bennett, G.J., Dantzer, R., Dougherty, P.M., Dunn, A.J., Meyers, C.A., et al., 2003. Are the symptoms of cancer and cancer treatment due to a shared biologic mechanism? A cytokine-immunologic model of cancer symptoms. Cancer 97 (11), 2919–2925.

Collado-Hidalgo, A., Bower, J.E., Ganz, P.A., Cole, S.W., Irwin, M.R., 2006. Inflammatory biomarkers for persistent fatigue in breast cancer survivors. Clinical Cancer Research 12, 2759–2766.

Coussens, L.M., Werb, Z., 2002. Inflammation and cancer. Nature 420 (6917), 860–867.

Demark-Wahnefried, W., Morey, M.C., Clipp, E.C., Pieper, C.F., Snyder, D.C., Sloane, R., et al., 2003. Leading the way in exercise and diet (Project LEAD): intervening to improve function among older breast and prostate cancer survivors. Controlled Clinical Trials 24 (2), 206–223.

DeNardo, D.G., Coussens, L.M., 2007. Inflammation and breast cancer. Balancing immune response: crosstalk between adaptive and innate immune cells during breast cancer progression. Breast Cancer Research 9 (4), 212.

Dobnig, H., Pilz, S., Scharnagl, H., Renner, W., Seelhorst, U., Wellnitz, B., et al., 2008. Independent association of low serum 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D levels with all-cause and cardiovascular mortality. Archives of Internal Medicine 168 (12), 1340–1349.

Fagundes, C.P., Murray, D.M., Hwang, B.S., Gouin, J.P., Thayer, J.F., Sollers 3rd, J., et al., 2011. Sympathetic and parasympathetic activity in cancer-related fatigue: More evidence for a physiological substrate in cancer survivors. Psychoneuroendocrinology 36, 1137–1147.

Ganz, P.A., Desmond, K.A., Leedham, B., Rowland, J.H., Meyerowitz, B.E., Belin, T.R., 2002. Quality of life in long-term, disease-free survivors of breast cancer: a follow-up study. Journal of the National Cancer Institute 94 (1), 39–49.

Glaser, R., Jones, J.F., 1994. Herpesvirus infections. M. Dekker, New York.

Glaser, R., Kiecolt-Glaser, J.K., 1994. Stress-associated immune modulation and its implications for reactivation of latent herpesviruses. In: Glaser, R., Jones, J. (Eds.), Human Herpesvirus Infections. Dekker, New York, pp. 245–270.

Glaser, R., Kiecolt-Glaser, J.K., 2005. Stress-induced immune dysfunction: implications for health. Nature Reviews Immunology 5, 243–251.

Glaser, R., Kiecolt-Glaser, J.K., Speicher, C.E., Holliday, J.E., 1985. Stress, loneliness, and changes in herpesvirus latency. Journal of Behavioral Medicine 8 (3), 249–260.

Glaser, R., Litsky, M.L., Padgett, D.A., Baiocchic, R.A., Yang, E.V., Chen, M., et al., 2006. EBV-encoded dUTPase induces immune dysregulation: implications for the pathophysiology of EBV-associated disease. Virology 346, 205–218.

Glaser, R., Padgett, D., Litsky, M., Baiocchi, R., Yang, E., Chen, M., et al., 2005a. Stress-associated changes in the steady-state expression of latent Epstein–Barr virus: implications for chronic fatigue syndrome and cancer. Brain, Behavior, and Immunity 19 (2), 91–103.

Glaser, R., Padgett, D.A., Litsky, M.L., Baiocchi, R.A., Yang, E.V., Chen, M., et al., 2005b. Stress-associate changes in the steady-state expression of latent Epstein–Barr virus: implications for chronic fatigue syndrome and cancer. Brain, Behavior, and Immunity 19, 91–103.

Jacobsen, P.B., Donovan, K.A., Vadaparampil, S.T., Small, B.J., 2007. Systematic review and meta-analysis of psychological and activity-based interventions for cancer-related fatigue. Health Psychology 26 (6), 660–667.

Jacobsen, P.B., Hann, D.M., Azzarello, L.M., Horton, J., Balducci, L., Lyman, G.H., 1999. Fatigue in women receiving adjuvant chemotherapy for breast cancer: characteristics, course, and correlates. Journal of Pain and Symptom Management 18 (4), 233–242.

Kiecolt-Glaser, J.K., Garner, W., Speicher, C., Penn, G.M., Holliday, J., Glaser, R., 1984. Psychosocial modifiers of immunocompetence in medical students. Psychosomatic Medicine 46 (1), 7–14.

Kiecolt-Glaser, J.K, Glaser, R., 1987. Psychosocial influences on herpesvirus latency. In E. Kurstak, Z. J. Lipowski, & P. V. Morozov (Eds.), *Viruses, Immunity, and Mental Disorders* (pp. 403–411). New York: Plenum.

Klemola, E., von Essen, R., Henle, G., Henle, W., 1970. Infectious-mononucleosis-like disease with negative heterophil agglutination test. Clinical features in relation to Epstein–Barr virus and cytomegalovirus antibodies. The Journal of Infectious Diseases, 608–614.

Koenig, W., 2003. Update on C-reactive protein as a risk marker in cardiovascular disease. Kidney international 63, S58–S61.

Kuo, C.P., Wu, C.L., Ho, H.T., Chen, C.G., Liu, S.I., Lu, Y.T., 2008. Detection of cytomegalovirus reactivation in cancer patients receiving chemotherapy. Clinical Microbiology and Infection 14 (3), 221–227.

Lawrence, D.P., Kupelnick, B., Miller, K., Devine, D., Lau, J., 2004. Evidence report on the occurrence, assessment, and treatment of fatigue in cancer patients. Journal of the National Cancer Institute Monographs 32, 40–50.

Libby, P., Ridker, P.M., Maseri, A., 2002. Inflammation and atherosclerosis. Circulation 105 (9), 1135.

Liu, L.Q., Fiorentino, L., Natarajan, L., Parker, B.A., Mills, P.J., Sadler, G.R., et al., 2009. Pre-treatment symptom cluster in breast cancer patients is associated with worse sleep, fatigue and depression during chemotherapy. Psycho-Oncology 18 (2), 187–194.

MacKinnon, D.P., Lockwood, C.M., Hoffman, J.M., West, S.G., Sheets, V., 2002. A comparison of methods to test mediation and other intervening variable effects. Psychological Methods 7, 83–104l.

MacKinnon, D.P., Lockwood, C.M., Williams, J., 2004. Confidence limits for the indirect effect: distribution of the product and resampling methods. Multivariate Behavioral Research 39 (1), 99–128.

Matalka, K.Z., Sidki, A., Abdul-Malik, S.M., Thewaini, A.J., 2000. Academic stress—Influence on Epstein–Barr virus and Cytomegalovirus reactivation, cortisol, and prolactin. Lab Medicine 31 (3), 163.

Mehta, S.K., Pierson, D.L., 2007. Reactivation of latent herpes viruses in cosmonauts during a soyuz taxi mission. Microgravity Science and Technology 19 (5), 215–218.

Mols, F., Vingerhoets, A.J.J.M., Coebergh, J.W., van de Poll-Franse, L.V., 2005. Quality of life among long-term breast cancer survivors: a systematic review. European Journal of Cancer 41 (17), 2613–2619.

Morin, C.M., 1993. Insomnia: Psychological assessment and management. Guilford Press, New York.

Nazmi, A., Diez-Roux, A.V., Jenny, N.S., Tsai, M.Y., Szklo, M., Aiello, A.E., 2010. The influence of persistent pathogens on circulating levels of inflammatory markers: a cross-sectional analysis from the multi-ethnic study of atherosclerosis. BMC Public Health 10, 706.

Nieboer, P., Buijs, C., Rodenhuis, S., Seynaeve, C., Beex, L.V.A.M., van der Wall, E., et al., 2005. Fatigue and relating factors in high-risk breast cancer patients treated with adjuvant standard or high-dose chemotherapy: a longitudinal study. Journal of Clinical Oncology 23 (33), 8296–8304.

Pawelec, G., Derhovanessian, E., Larbi, A., Strindhall, J., Wikby, A., 2009. Cytomegalovirus and human immunosenescence. Reviews in Medical Virology 19 (1), 47–56.

Polsky, D., Doshi, J.A., Marcus, S., Oslin, D., Rothbard, A., Thomas, N., et al., 2005. Long-term risk for depressive symptoms after a medical diagnosis. Archives of Internal Medicine 165, 1260–1266.

Radloff, L.S., 1977. The CES-D scale: a self-report depression scale for research in the general population. Applied Psychological Measurement 1, 385–401.

Reich, M., Lesur, A., Perdrizet-Chevallier, C., 2008. Depression, quality of life and breast cancer: a review of the literature. Breast Cancer Research and Treatment 110 (1), 9–17.

Roberts, E.T., Haan, M.N., Dowd, J.B., Aiello, A.E., 2010. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. American Journal of Epidemiology 172 (4), 363.

Savard, M., Savard, J., Simard, S., Ivers, H., 2005. Empirical validation of the Insomnia Severity Index in cancer patients. Psycho-Oncology 14, 429–441.

Staras, S.A., Dollard, S.C., Radford, K.W., Flanders, W.D., Pass, R.F., Cannon, M.J., 2006. Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. Clinical Infectious Diseases 43 (9), 1143–1151.

Steptoe, A., Gylfe, Å., Shamaei-Tousi, A., Bergstrom, S., Henderson, B., 2009. Pathogen burden and cortisol profiles over the day. Epidemiology and infection 137 (12), 1816–1824.

Steptoe, A., Shamaei-Tousi, A., Gylfe, A., Henderson, B., Bergstrom, S., Marmot, M., 2007. Socioeconomic status, pathogen burden and cardiovascular disease risk. Heart 93 (12), 1567–1570.

Van Der Ven, A., Van Diest, R., Hamulyak, K., Maes, M., Bruggeman, C., Appels, A., 2003. Herpes viruses, cytokines, and altered hemostasis in vital exhaustion. Psychosomatic Medicine 65, 194–200.

Ware, J.E., Sherbourne, C.D., Davies, A.R., 1992. Developing and testing the MOS 20-item short-form health survey: a general population application. In: Stewart, A.L., , Ware, J.E., Jr. (Eds.), Measuring Functioning and Well-Being. Duke University Press, Durham and London.

WHO. (2008). World Health Organization: Immunization, vaccines and biologicals – Epstein–Barr virus. Retrieved Feb 1, 2011, from http://www.who.int/immunization/topics/epstein_barr/en/index.html.

Wu, S.M., Andersen, B.L., 2010. Stress generation over the course of breast cancer survivorship. Journal of Behavioral Medicine 33 (3), 250–257.

Yang, E.V., Webster Marketon, J.I., Chen, M., Lo, K.W., Kim, S., Glaser, R., 2010. Glucocorticoids activate Epstein Barr virus lytic replication through the upregulation of immediate early BZLF1 gene expression. Brain, Behavior, and Immunity 24 (7), 1089–1096.