Published in final edited form as:

Biol Res Nurs. 2014 October ; 16(4): 429–437. doi:10.1177/1099800413519494.

Baseline Immune Biomarkers as Predictors of MBSR(BC) Treatment Success in Off-Treatment Breast Cancer Patients

Richard R. Reich, PhD^{1,2}, Cecile A. Lengacher, RN, PhD, FAAN^{2,3}, Kevin E. Kip, PhD³, Steven C. Shivers, PhD⁴, Michael J. Schell, PhD^{2,5}, Melissa M. Shelton, RN, PhD³, Raymond H. Widen, PhD⁶, Catherine Newton, MS⁵, Michelle K. Barta, PharmD³, Carly L. Paterson, RN, MS³, Jerrica R. Farias, RN, BS³, Charles E. Cox, MD^{4,5}, and Thomas W. Klein, PhD⁵

¹College of Arts and Sciences, University of South Florida Sarasota-Manatee, Sarasota, FL, USA

²H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL, USA

³College of Nursing, University of South Florida, Tampa, FL, USA

⁴University of South Florida Breast Health Clinical and Research Integrated Strategic Program, Tampa, FL, USA

⁵Morsani College of Medicine, University of South Florida, Tampa, FL, USA

⁶Tampa General Hospital, Tampa, FL, USA

Abstract

Researchers focused on patient-centered medicine are increasingly trying to identify baseline factors that predict treatment success. Because the quantity and function of lymphocyte subsets change during stress, we hypothesized that these subsets would serve as stress markers and therefore predict which breast cancer patients would benefit most from mindfulness-based stress reduction (MBSR)-facilitated stress relief. The purpose of this study was to assess whether baseline biomarker levels predicted symptom improvement following an MBSR intervention for breast cancer survivors (MBSR[BC]). This randomized controlled trial involved 41 patients assigned to either an MBSR(BC) intervention group or a no-treatment control group. Biomarkers were assessed at baseline, and symptom change was assessed 6 weeks later. Biomarkers included common lymphocyte subsets in the peripheral blood as well as the ability of T cells to become activated and secrete cytokines in response to stimulation with mitogens. Spearman correlations were used to identify univariate relationships between baseline biomarkers and 6-week improvement of symptoms. Next, backward elimination regression models were used to identify the strongest predictors from the univariate analyses. Multiple baseline biomarkers were significantly positively related to 6-week symptom improvement. The regression models identified B-lymphocytes and interferon- γ as the strongest predictors of gastrointestinal improvement ($p < .$

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Corresponding Author: Cecile A. Lengacher, RN, PhD, FAAN, College of Nursing, University of South Florida, MDC22, 12901 Bruce B. Downs Blvd., Tampa, FL 33612, USA. clengach@health.usf.edu.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

01), +CD4+CD8 as the strongest predictor of cognitive/psychological (CP) improvement ($p = .02$), and lymphocytes and interleukin (IL)-4 as the strongest predictors of fatigue improvement ($p < .01$). These results provide preliminary evidence of the potential to use baseline biomarkers as predictors to identify the patients likely to benefit from this intervention.

Keywords

mindfulness-based stress reduction; breast cancer; biomarkers

Following breast cancer treatment, women are at high risk for residual psychological symptoms of stress, anxiety, depression, fear of recurrence, and impaired cognitive functioning as well as physical symptoms of pain, fatigue (FA), and sleep disturbances, all of which can negatively impact quality of life after treatment (Dodd, Cho, Cooper, & Miaskowski, 2010; Von Ah, Habermann, Carpenter, & Schneider, 2013). These symptoms tend to cluster together and may have natural associations, similar shared pathways, and common underlying mechanisms (Dodd et al., 2010). In a study specific to breast cancer patients, Lengacher, Reich et al. (2012) identified three-symptom clusters among breast cancer patients: gastrointestinal (GI), cognitive/psychological (CP), and FA. They also demonstrated that a mindfulness-based stress reduction (MBSR) program (specific to breast cancer; MBSR intervention for breast cancer survivors, MBSR[BC]) was effective in reducing some symptoms. MBSR has been demonstrated to be a robustly efficacious treatment for relief of both psychological and physical symptoms in numerous conditions (Bohlmeijer, Prenger, Taal, & Cuijpers, 2010; Grossman, Niemann, Schmidt, & Walach, 2004; Hofmann, Sawyer, Witt, & Oh, 2010; Ledesma & Kumano, 2009).

As with most psychotherapeutic treatment studies, however, not all MBSR(BC) patients studied by Lengacher et al. (2012) experienced symptom reduction. This variability in results prompted an interest in identifying important patient characteristics that anteceded MBSR(BC) treatment and might help to predict its effects. Discovering baseline markers that identify patients most likely to benefit from treatment has long been a goal of “patient-centered” medicine (Simon, 2008). These identified markers increase the chances that patients will get the treatments that benefit them while decreasing the unnecessary burden of treatments unlikely to work. Typically, in previous patient-centered approaches, investigators have explored biological markers as predictors of medical treatment success (Wistuba, Gelovani, Jacoby, Davis, & Herbst, 2011), while psychosocial factors have been used to predict psychotherapeutic treatment success (Project MATCH Research Group, 1998). In the present study, we took a novel approach by examining baseline immunological biomarkers as predictors of which women who had recently completed breast cancer treatment would most benefit from a psychotherapeutic treatment.

We selected immunological biomarkers because (a) changes in these markers are related to stress (Graham, Christian, & Kiecolt-Glaser, 2006), (b) they have extensive links with symptoms of cancer and cancer treatment (detailed below), and (c) they are easily obtainable. Several biomarkers have been correlated with both physical and psychological symptoms experienced by cancer patients on and off treatment (Gardner, 1999; Kang et al.,

2009; van der Most, Currie, Robinson, & Lake, 2006). Changes in immune function and associated bio-marker expression in cancer survivors resulting from psychological distress may directly or indirectly affect survival outcomes (Andersen et al., 2007; Sephton et al., 2009). Additionally, chemotherapy (CT) and radiation therapy (RT) may affect immune recovery and survival outcomes (Kang et al., 2009). RT significantly reduces the levels of white blood cells, lymphocytes, and natural killer (NK) cell activity (Koukourakis, Zabatis, Zacharias, & Koukourakis, 2009; Standish et al., 2008; Yamazaki et al., 2002). Moreover, alterations in immune biomarkers have been shown to persist following treatment (Kang et al., 2009; Mozaffari et al., 2009; Standish et al., 2008). Among breast cancer patients receiving CT and RT, Standish et al. (2008) found decreases in the levels of lymphocytes, NK cell activity, monocyte phagocytic activity, and TNF- α production following RT compared to post-CT; interferon- γ (IFN- γ), however, was unchanged. Among 80 early-stage breast cancer patients who had completed treatment up to 1 year prior to the study, Kang et al. (2009) found that interleukin (IL)-6 and most cluster of differentiation (CD) cell subsets (except CD4+) recovered faster than did NK cell activity, levels of IL-2, IL-4, IFN- γ , and lymphocyte proliferation; CT alone or CT combined with RT significantly delayed IL-2 recovery (T helper (Th)1-type response), while IL-4 (Th2-type response) recovery was significantly delayed in the RT-only group. Studies have also shown that NK cell number and activity and T-cells are reduced following breast cancer treatment (Mozaffari et al., 2007; Solomayer et al., 2003). Mozaffari et al. (2007, 2009) found a lower number of CD4+ cells and lower expression of CD28+ on T cells during extended follow-up for breast cancer patients who received RT and CT; however, IFN- γ , IL-2, and IL-4 were higher in RT and CT patients versus RT-only patients.

The purpose of the present study was to identify baseline immune subsets, as markers of stress, that could be used to indicate patients who would most benefit from MBSR(BC) before the onset of treatment. This study can be considered a “developmental” study, as designated by Simon (2008), because the goal was to identify, in an exploratory manner, candidate bio-markers from a small sample. As such, it is the first step on the path to “validation” studies with specific hypotheses regarding specific biomarkers. Although this study may be considered developmental, it is not completely exploratory, as we chose the biomarkers to measure based on previously demonstrated links to physical and psychological symptoms following cancer treatment (Bower et al., 2009; Wang et al., 2012).

Materials and Methods

Sample and Setting

We recruited 41 female breast cancer survivors, diagnosed with Stage 0, I, II, or III breast cancer, who had undergone surgery (lumpectomy) and received adjuvant RT or RT and CT from the Moffitt Cancer Center. All women had completed treatment 2–12 weeks prior to enrollment. Women were excluded if they (a) had been diagnosed with Stage IV breast cancer, (b) had been treated for a breast cancer recurrence, (c) had undergone a mastectomy, or (d) had severe psychiatric problems. The Institutional Review Board at the University of South Florida in Tampa, Florida, approved the study protocol. Participants received US\$100 for participation, US\$50 at the beginning and US\$50 at the completion of the study.

Study Design and Random Assignment

Using a two-armed randomized controlled design, we randomly assigned participants to either a 6-week MBSR(BC) program or a wait-listed usual care (UC) group. Women in the UC group could elect to receive the MBSR(BC) intervention after 6 weeks (upon study completion). Subjects were stratified by stage of cancer (0, I, II, or III) and type of treatment (RT alone or RT and CT).

Data Collection Procedures

At the orientation session, all participants provided written informed consent, and we collected baseline data on demographics and symptoms. In the MBSR(BC) group, we collected blood for immune analyses at the orientation session prior to the start of the intervention and again at a follow-up session for MBSR(BC) participants within 2 weeks of completion of the 6-week intervention. In the UC group, we collected data and blood samples at baseline during the orientation session and again 6–8 weeks from baseline at a follow-up session for UC participants. We also collected data on symptoms for the MBSR(BC) and UC groups at their respective follow-up sessions.

Intervention and UC

MBSR(BC) is a 6-week program adapted from Kabat-Zinn's original 8-week MBSR program (Kabat-Zinn, Lipworth, & Burney, 1985) to address concerns of breast cancer survivors by training them to use mindfulness and attention to adapt to emotional/psychological symptoms (anxiety, depression, and fear of recurrence) and physical symptoms such as FA, pain, and sleep disturbance. A psychologist trained in MBSR taught the weekly 2-hr sessions. Participants received a manual that guided the classes. The MBSR(BC) program includes three components: (a) educational materials and exercises related to meditation practices and the mind–body connection, (b) practice time and a CD on which verbal support for four meditative practices was recorded (sitting meditation, walking meditation, body scan, and gentle hatha yoga), and (c) opportunity for group discussion, including time to answer questions related to barriers to formal and informal practice. Participants were asked to spend 15–45 min daily outside of the group sessions on formal and informal practice and to record their practice time in a diary. Formal mindfulness practice includes sitting meditation, walking meditation, body scan, and yoga practice. Informal mindfulness meditation practices, or mindfulness in everyday life, incorporate an awareness of pleasant and unpleasant events and encourage awareness of routine activities.

The UC group had standard posttreatment clinic visits. We specifically asked women in this group not to use or practice meditation, yoga techniques, or MBSR during the study. Following completion of the study, we provided each UC participant with a brief orientation to MBSR(BC) along with a manual and CDs of the complete program and offered them optional scheduled classes.

Measures

Symptoms—We measured symptoms using the M. D. Anderson Symptom Inventory (MDASI; Cleeland et al., 2000). The MDASI measures the severity of symptoms and the

degree to which they interfere with daily functioning. The instrument includes 2 items for each symptom, one reflecting the last 24 hours and the other the last 7 days. Respondents are asked to rate the severity of 13 core symptoms from 0 (*not present*) to 10 (*pain as bad as you can imagine*). There are also 6 symptom interference items in which respondents rate the level at which symptoms interfere with function. Internal consistency has been shown to range from 0.82 to 0.94 (Cleeland et al., 2000). Use of the MDASI has been supported in studies using patients with multiple cancer diagnoses from multiple populations (Chen & Lin, 2007; Ivanova et al., 2005; Kwon et al., 2006; Okuyama et al., 2003; Tseng, Cleeland, Wang, & Lin, 2008; Wang et al., 2004, 2006). Using the “last 24 hr” items from the MDASI, Lengacher, Reich, et al. (2012) identified three central symptom clusters in breast cancer patients: (a) GI (dry mouth, nausea, vomiting, lack of appetite), (b) CP (trouble remembering, distress, and sadness), and (c) FA (disturbed sleep, and drowsiness). To calculate cluster scores, we categorized items by symptom cluster and summed responses to items included in each cluster.

Peripheral blood lymphocytes—Peripheral blood samples were collected between 9 a.m. and noon into heparinized tubes and processed immediately for lymphocyte analysis. Heparinized blood (0.1 ml) was mixed with 2.0 ml of IOTest 3 lysing solution (Beckman Coulter) to lyse red blood cells, and 1.0 ml of the mixture was counted in a Vi-Cell XR cell viability analyzer (Beckman). The number of viable cells per microliter (μ l) was calculated for each sample. The percentage of lymphocyte subpopulations (subsets) in peripheral blood samples was determined by antibody staining and flow cytometry as described previously (Lengacher et al., 2013). Briefly, heparinized blood samples were stained and incubated in two different tubes with antibodies (BD Bioscience) to either CD45, CD4, CD8, and CD3 (Tube 1) or CD45, CD19, CD3, and CD56 (Tube 2). In a third tube, cells were stained with antibodies (BD Bioscience) to the surface antigens CD3 and CD69 (an activation marker) and then processed with Cytofix/Cytoperm (BD Bioscience) for intracellular staining with antibodies to IFN- γ (Th1 T cells) and IL-4 (Th2 T cells).

The stained samples were then analyzed on an FACScalibur flow cytometer (Beckton Dickinson) using MultiSet software, and the percentages of CD3+, CD4+, and CD8+ T cells, CD19+ B cells, and CD56+ NK cells were determined. The absolute number of cells in each subset was determined using these percentages combined with the number of viable white blood cells determined as described previously.

The percentages of Th1 (CD3+CD4+IFN- γ +) and Th2 (CD3+CD4+IL-4+) subsets were also determined on patient blood samples stimulated with two different mitogens and stained for intracellular cytokines as described previously (Lengacher et al., 2013). Briefly, blood samples were incubated for 1 hr with either phorbol myristate acetate (5 ng/ml, Sigma Chemical) and ionomycin (500 ng/ml, Sigma) or with PHA (phytohemagglutinin; 20 μ g, Sigma) and then incubated for an additional 4 hr with mitogen and brefeldin A (1 μ g/ml, BD Bioscience) to prevent secretion of cytokines from the stimulated cells and thus build up the intracellular levels of IFN- γ and IL-4. Next, the cells were stained with antibodies (BD Bioscience) to the surface antigens CD3 and CD69 (an activation marker) and then processed with Cytofix/Cytoperm (BD Bioscience) for intracellular staining with antibodies to IFN- γ (Th1 T cells) and IL-4 (Th2 T cells). Stained cells were analyzed by flow

cytometry and Cellquest software (BD Bioscience) for CD3+CD69+ cells that were also either IFN- γ + or IL-4+.

Demographics, medical history—We administered standard demographic data and detailed clinical history forms at baseline and recorded any changes to the data at 6 weeks.

Statistical Analyses

Baseline characteristics (demographics and immune biomarker levels) were compared between MBSR(BC) and UC conditions using the χ^2 test or Wilcoxon rank sum test (for categorical and continuous variables, respectively). Pre–post symptom differences were analyzed using the Wilcoxon signed ranks test. To calculate symptom change, posttreatment symptom cluster scores were subtracted from pretreatment scores.

The analyses to identify baseline biomarkers that predicted 6-week symptom cluster change were run for both MBSR(BC) and UC patients to determine whether baseline immune biomarkers predicted improvement in general or only MBSR(BC)-specific improvement. Identifying baseline bio-markers that predicted 6-week change in symptom clusters was a two-step process. First, bivariate Spearman correlations were calculated between symptom cluster change scores and each baseline biomarker value. Spearman correlations were chosen because of the tendency for the biomarkers not to be normally distributed (most were highly skewed). Because these initial steps to build prediction models were designed to catch all relevant variables, candidate predictors were identified with the relatively liberal p value $< .10$ (Hosmer & Lemeshow, 2000). Second, to identify the strongest predictors (those that explained the most independent variance in symptom change scores), a backward elimination regression model was created for each of the symptom clusters. To reduce the aforementioned problem of skew in the biomarkers, each was log and square root transformed. The transformation yielding the lowest skew was chosen for the regression models. All candidate bio-markers were entered in the models and subsequently removed if their p value was greater than .05. Cancer treatment (RT or RT and CT) and time since cancer treatment also were entered into the regression models as covariates because previous studies have demonstrated differential immune biomarker responses by cancer treatment (Kang et al., 2009; Koukourakis et al., 2009; Mozaffari et al., 2007, 2009; Solomayer et al., 2003; Standish et al., 2008; Yamazaki et al., 2002).

Results

Demographic characteristics and immune biomarkers did not differ between UC and MBSR(BC) groups at baseline (Tables 1 and 2). Also, the baseline biomarker levels we obtained in the present study are similar to those other researchers have reported for similar populations. For instance, the number of lymphocytes averaged approximately 1,000/ μ l of blood (see Table 2), which is within the range of 490–6,000/ μ l (median of 2,000/ μ l) as reported by Park and Han (2012). The percentages of CD3+ lymphocytes (~80%), CD16+/CD56+ cells (~10%), and CD19+ cells (~10%) were also in line with other reports (Abud-Mendoza et al., 2012; Carlson, Specia, Faris, & Patel, 2007; Park & Han, 2012). Regarding the ratio of Th1 to Th2 cells, others have reported ratios from 12.5 (Toldi et al., 2011) to 5.1 (Cseh et al., 2012), while we observed ratios ranging from 3.4 to 9.1 in the present study,

depending upon the group. So, although all the women in the study received either RT only or RT combined with CT, participants as a whole showed lymphocyte subset values within the normal range upon entering the study, and analysis of the baseline data by cancer treatment type showed no significant differences.

Symptom cluster scores tended to go down across both UC and MBSR(BC) groups for all three clusters (Table 3). These within-group decreases were significant, however, only in the MBSR(BC) condition for the fatigue cluster ($p = .003$) and in the GI cluster ($p = .035$).

Although groups did not differ on mean baseline biomarker levels, we did note substantial within-group variability, as indicated by the standard deviations (Table 2). We assessed the relationship between this variability and 6-week symptom change scores using Spearman correlations. For the UC group, two bio-markers were significant univariate predictors of improvement in the GI symptom cluster (number of CD4+CD8+ T cells; PHA-stimulated CD3+ [% of lymphocytes]), but none were predictors of improvement in the other clusters. For the MBSR(BC) group, five biomarkers were significant univariate predictors of GI symptom cluster improvement, one was a significant predictor of CP symptom cluster improvement, and eight were significant predictors of FA symptom cluster improvement (Table 4). Although the CP cluster had only one significant predictor, number of CD4+CD8+ T cells, the correlation between this predictor and cluster ($r = .61$) was the strongest when compared to all other predictors.

Our initial plan was to introduce all biomarkers that had demonstrated significant univariate prediction capability into the multivariate regression models predicting symptom cluster improvement following MBSR(BC). The fact that several of the biomarkers were highly intercorrelated, however, introduced the potential problem of multicollinearity into our models. Therefore, when two biomarkers were highly correlated with each other ($r > .50$), we introduced only the strongest predictor into the model. For the GI symptom cluster, two predictors remained statistically significant following the backward elimination procedure: B lymphocytes and PHA-stimulated CD3+, IFN- γ + [Th1]. The R^2 for this two-predictor model was .65, meaning the baseline values of these two biomarkers accounted for 65% of the variance in GI symptom cluster change (Table 4). Two predictors also remained statistically significant for the FA cluster—total lymphocytes and PHA-stimulated CD3+, IL-4+ (Th2)—with an R^2 value of .56. For the CP cluster, one predictor remained statistically significant—CD4+ CD8+—with an R^2 of .31. Cancer treatment and time since cancer treatment, included as covariates in these regression models, were not significant predictors of symptom cluster improvement in any of the three regression models. As a result, we removed these variables in the backward elimination regression procedure. Final regression models are summarized in Table 5.

Discussion

The findings of the present study introduce the possibility of predicting the success of MBSR(BC) treatment using biomarker values obtained at the time of study entry. The central purpose of the study was to identify baseline immune subsets, as markers of stress, that could be used to indicate patients who would most benefit from MBSR(BC) before the

onset of treatment. Our study concentrated on lymphocyte subpopulations as biomarkers among two groups of female breast cancer survivors, one of which received UC while the other participated in an MBSR(BC) program. Despite the similarity in baseline measures between the groups, substantial individual variation was apparent in the data. We tested these variations as predictors of MBSR(BC) symptom reduction in a sample where MBSR(BC) treatment had already demonstrated efficacy (Lengacher, Kip, et al., 2012).

Several biomarkers were significant univariate predictors of symptom cluster improvement, such as higher values for total lymphocytes, T cells, B cells, and CD4+CD8+ precursor cells as well as increased potential to respond to a relatively weak stimulus like PHA (Table 3). Of these, CD3+, CD4+CD8+, and total lymphocytes were significant univariate predictors for more than one cluster (e.g., CD4+CD8+ cells were associated with all three-symptom clusters; Table 3). After using backward elimination procedures, we developed multivariate regression models (Table 4) to define the cell subsets that were the strongest independent predictors for improvement in each symptom cluster after MBSR(BC). However, we did not find any significant predictors that were common across all of the symptom clusters.

At this point, it is only possible to speculate as to why different subsets should uniquely predict improvement in a given symptom cluster. Perhaps all the subsets we have observed to have significant associations with symptom cluster improvement are representative of a recovering immune response, from increased total lymphocytes and CD4+CD8+ naive cells to increases in circulating B cells and increases in T cells capable of responding to PHA. Thus, it is possible that the immunity of some cancer patients recovers better than that of others, rendering them better able to benefit from MBSR. This recovering immune response could result in both elevated B lymphocytes in peripheral blood and increases in the ability to produce the Th1 cytokine IFN- γ after stimulation of the patient's T cells in vitro in the presence of underlying infections against which antibodies are being produced. It makes sense that patients experiencing GI symptoms, especially nausea and vomiting, would have elevations in immunologic cells associated with antibody production. Breast cancer patients who have undergone RT and/or CT often have such symptoms that are related to sensitivity of the cells lining the GI tract to these agents and/ or an underlying immune dysregulation (Andreyev, Davidson, Gillespie, Allum, & Swarbrick, 2012).

The number of baseline CD4+CD8+ T cells was associated with some type of improvement for all three of the defined symptom clusters (Table 3). These double-positive cells with cell-surface markers that are normally mutually exclusive are usually detected only in very small numbers in the peripheral blood (Parel & Chizzolini, 2004). Investigators have observed increases in CD4+CD8+ T cells in patients under a variety of immunologic stresses, such as chronic viral infections (Nascimbeni, Pol, & Saunier, 2011; Nascimbeni, Shin, Chiriboga, Kleiner, & Rehmann, 2004; Weiss et al., 1998) and autoimmune diseases (Parel & Chizzolini, 2004). Although the origin and function of CD4+CD8+ T cells in the peripheral blood remains controversial, there is some evidence that they represent mature antigen-specific effector memory cells that are involved in latent and/or active immune responses (Nascimbeni et al., 2011).

Our results also showed that, along with patients showing elevated levels of total lymphocytes in the peripheral blood, patients whose T cells were able to produce IL-4 (Th2 response) after stimulation in vitro derived the most benefit from MBSR(BC) with respect to fatigue symptoms. Collado-Hidalgo, Bower, Ganz, Cole, and Irwin (2006) demonstrated that decreased activated (CD69+) T lymphocytes in the peripheral blood is highly diagnostic of fatigue in breast cancer survivors. Other studies have demonstrated a bias toward Th2-type responses, particularly IL-4 production after polyclonal stimulation, in patients with chronic fatigue syndrome (Schwarz, Chiang, Muller, & Ackenheil, 2001; Torres-Harding, Sorenson, Jason, Maher, & Fletcher, 2008). Although the results of these studies are consistent with our findings, the mechanistic nature of the relationship between fatigue and Th2-type T cell responses is not clear, though our cumulative findings do suggest that a functional immune response is required.

For women receiving the MBSR(BC) program in the current study, the significant relationships between immunological bio-markers and symptom improvement were exclusively positive. This positive relationship means that the larger the baseline immune biomarker value, the greater the improvement in symptoms. In other words, it appears that symptom improvement was associated with increased immune activity at baseline.

Despite having received RT or RT and CT, all of the women showed lymphocyte subset values within the normal ranges upon entering the study, and analysis of the data by cancer treatment type showed no significant differences. Partially contributing to this normalcy was the fact that two thirds of the participants had completed treatment 1–3 months before the start of the study. A number of reports have examined immune markers as a function of immunosuppressive anticancer therapy (Mills et al., 2008). Most of these studies examined blood cytokine levels as biomarkers, and the constant theme was that cytokines increased during or shortly after treatment (Mills et al., 2008) but returned to pretreatment levels 1–3 months after treatment (Bower et al., 2009; Sepah & Bower, 2009; Wang et al., 2012).

The results of this study should be interpreted with caution due to the small sample size. The parent study included larger numbers, but we had previously shown that time since treatment enhanced the effect of MBSR(BC) on immune biomarker levels (Lengacher, Kip, et al., 2012). Therefore, we limited participation in the present study to patients who were recently off-treatment, greatly curtailing the sample. The large number of significant relationships between the biomarkers and symptom clusters, however, should provide sufficient evidence to move from this exploratory developmental approach to a larger validation approach (Simon, 2008).

If the predictive value of these biomarkers is validated, the cost-effectiveness of MBSR(BC) may be amplified as it could be targeted toward patients with high levels of these biomarkers, particularly within the first few months of recovery. Because MBSR has been successful across many patient populations (Bohlmeijer et al., 2010; Grossman et al., 2004; Hofmann et al., 2010; Ledesma & Kumano, 2009), future research should test the generalizability of these specific biomarker results.

Acknowledgments

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the National Institutes of Health, National Cancer Institute, grant number R21CA109168.

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Table 1

Demographic Characteristics of Female Breast Cancer Survivors at Baseline.

Variable	UC (<i>n</i> = 24)	MBSR(BC) (<i>n</i> = 17)	<i>p</i> value
Age, years (mean, <i>SD</i>)	58.2 (9.5)	58.0 (10.3)	.90
% minority ^a	29	18	.40
% married	50	77	.08
% employed	54	59	.44
Stage of disease %			.25
0	25	6	
I	50	71	
II	21	12	
III	4	12	
% who received chemotherapy ^b	30	35	.68

Note. MBSR(BC) = mindfulness-based stress reduction program for breast cancer survivors; UC = usual care.

^aThe category “minority” included White-Hispanic, Black non-Hispanic, Ashkenazi Jew, and Native American.

^bAll participants received radiation treatment.

Table 2Mean (*SD*) Levels of Biomarker Lymphocyte Subsets Among Female Breast Cancer Survivors at Baseline.

	Number of cells/ μ l of blood		
Biomarker	UC (<i>n</i> = 24)	MBSR(BC) (<i>n</i> = 17)	<i>p</i> value ^a
Lymphocyte subsets			
CD3+	815 (215.6)	809 (382.4)	.57
CD8+	247 (110.0)	271 (150.7)	.84
CD4+	554 (174.8)	527 (265.5)	.45
CD4+CD8+	11 (6.8)	13 (8.8)	.51
CD16+/CD56+ ratio (NK cells)	121 (74.0)	112 (57.0)	.72
CD19+ (B cells)	89 (37.4)	77 (52.5)	.19
Total lymphocytes	1054 (268.2)	1031 (450.8)	.44
CD4+/CD8+ ratio	2.7 (1.7)	2.3 (1.5)	.40
Mitogen-stimulated subsets ^b			
PHA stimulated			
CD3+	62 (11.4)	60 (14.5)	.97
CD3+CD69+ (activated)	31 (14.1)	29 (17.8)	.58
CD3+IFN- γ + (Th1)	11 (10.2)	12 (9.2)	.44
CD3+IL-4+ (Th2)	1.3 (1.13)	1.5 (1.42)	.85
PMA/IO stimulated			
CD3+	70 (11.4)	72 (9.8)	.62
CD3+CD69+ (activated)	51 (24.5)	52 (26.5)	.80
CD3+IFN- γ + (Th1)	22 (12.6)	21 (9.7)	.89
CD3+IL-4+ (Th2)	6.6 (6.8)	4.9 (4.1)	.72

Note. *SD* = standard deviation; CD = cluster of differentiation; IFN = interferon; IL = interleukin; MBSR(BC) = mindfulness-based stress reduction program for breast cancer survivors; NK cells = natural killer cells; PHA = phytohemagglutinin; PMA/IO = phorbol myristate acetate/ionomycin; Th1 and Th2 = T helper 1 and 2 response; UC = usual care.

^a All comparisons made using Wilcoxon rank-sum test.

^b All mitogen-stimulated subsets are percentage of T cells, with the exception of CD3+, which is percentage of lymphocytes.

Table 3

Symptom Cluster Scores at Baseline and 6 Weeks.

Symptom cluster (mean score) ^a	UC (<i>n</i> = 24)		MBSR(BC) <i>n</i> = 17	
	Baseline	6 weeks	Baseline	6 weeks
Gastrointestinal	6.0 (8.1)	5.5 (8.1)	4.5 (5.4)	1.9 (3.5) **
Cognitive/psychological	9.1 (8.7)	7.8 (8.5)	7.1 (10.1)	4.3 (5.1)
Fatigue	9.1 (7.1)	7.3 (6.0)	8.8 (6.9)	4.6 (5.3) *

Note. MBSR(BC) = mindfulness-based stress reduction program for breast cancer survivors.

^a Symptom-cluster scores were from the M. D. Anderson Symptom Inventory.

* Within-group difference ($p < .05$).

** Within-group difference ($p < .01$).

Table 4

Correlations (Spearman's ρ) Between Baseline Biomarkers and Symptom Cluster Improvement by Condition (Usual Care [UC; $n = 24$] vs. Mindfulness-Based Stressed Reduction for Breast Cancer Program [MBSR(BC); $n = 17$]) at 6 Weeks of Treatment Among Female Breast Cancer Survivors.

Biomarker	Symptom cluster					
	Gastrointestinal		Cognitive/psychological		Fatigue	
	UC	MBSR(BC)	UC	MBSR(BC)	UC	MBSR(BC)
Lymphocyte subsets (# of cells/ μ l of blood)						
CD3+	-.16	.44*	-.31	.31	.10	.58**
CD8+	.17	.38	.05	.38	-.01	.54**
CD4+	-.11	.37	-.26	.17	.17	.50**
CD4+CD8+	.40*	.14	-.10	.61***	.07	.44*
NK cells	-.02	.18	-.26	-.20	.00	.21
B lymphocytes	-.21	.44*	-.16	-.01	-.01	.34
Total lymphocytes	-.07	.42*	-.24	.27	.17	.58**
Mitogen-stimulated subsets ^a						
PHA stimulated						
CD3+	-.38*	.39	.13	.13	.00	.32
CD3+CD69+ (activated)	-.31	-.25	.00	.04	-.02	-.08
CD3+IFN- γ + (Th1)	.08	.53**	.14	.33	.01	.34
CD3+IL-4+ (Th2)	.18	.43	-.14	.27	.28	.54**
PMA and IO stimulated						
CD3+	-.22	.43*	-.08	.18	.07	.47*
CD3+CD69+ (activated)	.18	-.16	-.03	.26	.23	.08
CD3+IFN- γ + (Th1)	.12	.37	.04	.18	.03	.40
CD3+IL-4+ (Th2)	-.07	.37	-.02	.35	.22	.48*

Note. UC = usual care; MBSR(BC) = mindfulness-based stress reduction program for breast cancer survivors; CD = cluster of differentiation; IFN = interferon; IL = interleukin; NK cells = natural killer cells; PHA = phytohemagglutinin; PMA/IO = phorbol myristate acetate/ionomycin; Th1 & Th2 = T helper 1 or 2 response.

^a All mitogen-stimulated subsets are percentage of T cells, with the exception of CD3+, which is percentage of lymphocytes.

* $p < .10$.

.10' > *p*

'50' < *p*
**

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Table 5

Backward Elimination Regression Models Predicting Symptom Cluster Change in Female Breast Cancer Survivors Receiving Mindfulness-Based Stress Reduction for Breast Cancer (MBSR[BC]) Treatment.

Predictors ^a	<i>p</i> value	Model ^b <i>R</i> ²	Model <i>p</i> value
Gastrointestinal cluster			
B lymphocytes ^c	< .001		
PHA-stimulated CD3+IFN- γ +	.013		
(Th1) ^c		.65	<.001
Cognitive/psychological cluster			
CD4+CD8+	.012		
		.31	.012
Fatigue cluster			
Total lymphocytes ^d	.008		
PHA-stimulated CD3+IL-4+	.007		
(Th2) ^d		.56	.003

Note. CD = cluster of differentiation; IFN = interferon; IL = interleukin; PHA = phytohemagglutinin; Th1 & Th2 = T helper 1 or 2 response.

^aPredictors were retained in model if $p < .05$.

^bAdjusted R^2 .

^cLog transformed.

^dSquare root transformed.