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Examining the relationship between inflammatory markers and menopausal status in breast cancer patients undergoing chemotherapy treatment

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Examining the Relationship between Inflammatory Markers and Menopausal Status in Breast Cancer Patients Undergoing Chemotherapy Treatment

A Thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Biology by

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Committee in charge:
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Professor Susan Golden

2011
The Thesis of Nicolle Christina Ma is approved and it is acceptable in quality and form for publication on microfilm and electronically:

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Co-Chair

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Chair

University of California, San Diego

2011
Dedication

This thesis is dedicated to my parents. Thank you for all your love and support.
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Being part of the collaboration between two labs was a fantastic experience that would not have been possible without Dr. Sonia Ancoli-Israel, who I was lucky to have as my unofficial advisor. She welcomed me into her lab from the very beginning and allowed me to engage in every aspect of the chemobrain and cancer studies, whether it was inputting data, attending statistics meetings, conducting neuropsychological tests or performing data analysis. I am thankful for her guidance.

At the Medical Center in Hillcrest I would like to thank Barbara Woods, who ran every assay with me and taught me about lab work in general. Her patience and relaxed attitude created a wonderful learning environment. Thanks to Chris Pruitt, who first helped me get acquainted with the lab and helped me hone my laboratory techniques. I would also like to thank him for his wonderful conversations ranging from traveling to politics to pop culture.
At the Gillin Sleep and Chronomedicine Research Center I need to thank Michelle Rissling, who never hesitated to answer any of my questions. With her help I was able to learn about research design, statistical analysis, the chemobrain research study and the previous cancer study. I am grateful for the incredible amount of time she spent with me in person, on the phone and via e-mail. Many thanks to Sue Lawton, who helped me get acquainted with the chemobrain study, taught me how to conduct neuropsychological tests, graciously provided me with data and updated the data files I worked with. I also need to thank Dr. Loki Natarajan, who thoroughly checked and double checked all the statistical tests I ran and provided helpful advice on how to statistically analyze research data. Thanks to Ariel Neikrug, who gave me tips and advice on research design, literature research, statistical analysis and working with SPSS. I also need to thank Dr. Lianqi Liu, who showed me how to work with actiwatches, actigraphs and allowed me to attend and conduct PSG tests. I would also like to acknowledge Sherella Johnson, who gave me the opportunity to practice my first consent and organize the chemobrain patient and control data. Thanks to Dr. Lavinia Fiorentino, who helped me with canonical correlations and general strategies for how to work with SPSS.

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ABSTRACT OF THE THESIS

Examining the Relationship between Inflammatory Markers and Menopausal Status in Breast Cancer Patients Undergoing Chemotherapy Treatment

by

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Master of Science in Biology

University of California, San Diego 2011

Professor Paul Mills, Chair

The goal of this study was to determine whether menopausal status accounts for the variability in circulating levels of inflammatory biomarkers that have been observed in studies examining women with breast cancer undergoing treatment. The hypothesis was that women with breast cancer who are pre-menopausal will have higher levels of inflammation compared to women with breast cancer who are post-menopausal.

Data on inflammation (circulating levels of soluble Intercellular Adhesion Molecule-1 (sICAM-1), Vascular Endothelial Growth Factor (VEGF), Interleukin-6 (IL-6), C-Reactive Protein (CRP), Tumor Necrosis Factor-alpha (TNF-alpha) and P-
selectin) and psychosocial measurements were collected from 85 women with newly diagnosed breast cancer within a few days before starting chemotherapy and approximately 2.5 months later at week 3 of cycle 4 of chemotherapy. In addition, each study participant filled out questionnaires assessing menopausal status and symptom severity, which asked about regular menses before/after chemotherapy (pre-menopausal status), irregular menses before/after chemotherapy (peri-menopausal status), post-menses before/after chemotherapy (post-menopausal status) and post-hysterectomy or post-oophorectomy before/after chemotherapy. Based on responses to the menopausal questionnaires, women were divided into two categories: either pre/peri (N = 29), meaning that the women were pre-menopausal or peri-menopausal before chemotherapy and pre-menopausal or peri-menopausal after chemotherapy or peri/post (N = 56), meaning that the women were peri-menopausal or post-menopausal before chemotherapy and post-menopausal after chemotherapy.

Contrary to my hypothesis, multiple regression and canonical correlation statistical analyses indicated that menopausal status does not account for the variability observed in inflammatory biomarker data in women with breast cancer prior to or following chemotherapy.
Introduction

Breast Cancer Incidence

Every year, over 1 million women around the world contract breast cancer (Stuckey A., 2011). After cervical cancer, the leading cause of cancer death in women is breast cancer in developing countries (Lynch et al., 2011; National Cervical Cancer Coalition, 2010).

Other than non-melanoma skin cancer, breast cancer is the most common cancer among women in the United States (American Cancer Society, 2010; Centers for Disease Control and Prevention, 2011). As of January 2007, it was estimated that there were 2,591,855 women alive who had a history of breast cancer, including those with the disease and those cured of it (National Cancer Institute, 2010). One in eight women will be diagnosed with breast cancer during their lifetime based on rates from 2005-2007. The median age for breast cancer diagnosis was 61 years old from 2003-2007. Non-Hispanic white women from 1999-2007 had the highest incidence rate of breast cancer, with an incidence of 126.5 per 100,000 women (Centers for Disease Control and Prevention, 2010; Lythcott, 2003). Black women, with an incidence of 118.3 per 100,000 women, had the second highest incidence rate of breast cancer. Hispanic women had the third highest incidence rate of breast cancer, followed by Asian/Pacific Islander and American Indian/Alaska Native women. After adjusting for age and taking into account white, black, Asian/Pacific Islander, American
Indian/Alaska Native and Hispanic races and ethnicities, the incidence rate was 122.9 for every 100,000 women per year from 2003-2007 (National Cancer Insitute, 2010).

*Breast Cancer Associated Mortality*

After lung cancer, breast cancer in the United States is responsible for the most cancer deaths in women (American Cancer Society, 2010), with a median age of death at 68 years old (National Cancer Insitute, 2010). With an incidence of 32.4 per 100,000 women, black women have the highest mortality rate, followed by white women with an incidence of 23.4 per 100,000 women (Centers for Disease Control and Prevention, 2010; Lythcott, 2003). Hispanic women had the third highest mortality rate followed by American Indian/Alaska Native and Asian/Pacific Islander.

From 1999-2006, the five-year relative survival by stage at diagnosis was 98% for the 60% of women who had a localized stage (National Cancer Insitute, 2010). There was an 83.6% five-year relative survival for the 33% of women who had a regional stage (the cancer had spread to the lymph nodes). There was a 23.4% five-year relative survival for the 5% of women who had metastasized cancer. There was a 57.9% five-year relative survival for the 2% of women who had an unknown stage at diagnosis.

*Breast Cancer Treatment*

Breast cancer can be treated in many ways depending on the stage of the disease. For a specific tumor, local treatment such as radiation or surgery is used so
that the rest of the body is unaffected (American Cancer Society, 2011). Systemic treatment such as chemotherapy, hormone therapy and targeted therapy is given orally or injected into the bloodstream to target cancer cells that have possibly spread beyond the breast. Neoadjuvant therapy is systemic treatment (usually chemotherapy) that is given before surgery in order to shrink the size of a tumor. Adjuvant therapy is additional treatment that is given after surgery.

Research studies have examined predictors of how women respond to chemotherapy. One study found that health related quality of life had statistically significant relationships with response to treatment, social functioning, role functioning, fatigue, appetite loss and nausea/vomiting (Svensson et al., 2011). Health related quality of life variables can thereby potentially serve as predictors of response to treatment in women with metastatic breast cancer. Another predictor is protein 53 (p53) which acts as a tumor suppressor and has a statistically significant relationship with markers of aggressive tumor biology (Guarneri et al., 2010). Women with atypical ductal hyperplasia and atypical lobular hyperplasia who drink alcohol and have a first degree relative with breast cancer have an increased risk of the disease as opposed to those who do not have an atypical breast cancer (Whiffen et al., 2011). In women with triple-negative tumors, expression of cytokeratin seems to indicate disease-free survival (Mehta et al., 2010).
Another predictor of survival is circulating levels of inflammatory biomarkers that are indicative of inflammation. In advanced non-small cell lung cancer, for example, elevations in inflammatory cytokines are significantly associated with worsening of symptoms in patients undergoing chemoradiation therapy (Wang et al., 2010). In breast cancer, inflammation increases as the stage of the disease gets worse (Pierce et al., 2009). Both experimental and clinical data show that the kind of immune response elicited determines its outcome in response to an evolving breast neoplasm (DeNardo and Coussens, 2007). Primary breast tumors are not associated with significant inflammation while metastasis is associated with chronic inflammation (Pierce et al., 2009). Both experimental and clinical data indicate that chronic inflammation increases mammary tumor development via activation of the innate and adaptive immune system (Pierce et al., 2009). Pro-inflammatory cytokines can ease tumor growth and metastasis via activating vascular endothelial cells, fibroblasts and tumor-associated macrophages in the tumor microenvironment as well as by altering the tumor cell biology (Cole, 2009). Systemic inflammation may reactivate dormant tumors or lead to the growth of micrometastases (Cole, 2009). Breast cancer patients in stage 4 of the disease have significantly higher levels of SAA (serum amyloid A) and CRP (C-reactive protein) as compared to controls (O'Hanlon et al., 2002). While increased pre-treatment levels of CRP have been linked to a decrease in survival in metastatic breast cancer patients, CRP levels are not found to be related to survival in patients with primary breast cancer (Pierce et al., 2009).
Chronic inflammation may elevate the risk that breast cancer will recur (Pierce et al., 2009).

There have been studies looking at inflammation and its association to psychosocial factors in breast cancer patients. One study, for example, looked at the relationship between inflammatory markers and fatigue (Collado-Hidalgo et al., 2006). In this study, IL-6 was significantly increased in breast cancer patients two years after chemotherapy, whereas another study that examined inflammation, fatigue and quality of life in breast cancer patients at cycle 1 and cycle 4 of anthracycline based chemotherapy did not find IL-6 levels to be related to fatigue (Mills et al., 2004). The latter study, however, found that there was a statistically significant increase in VEGF and sICAM-1 during anthracycline based chemotherapy (cycle 4 week 1) compared to before chemotherapy (Mills et al., 2004). There are data to indicate that IL-6 can mediate angiogenesis directly by affecting endothelial cell proliferation and indirectly by increasing the VEGF load in megakaryocytes (Salgado et al., 2003). Another study looking at inflammatory markers in response to anthracycline based chemotherapy for breast cancer found elevations in VEGF, sICAM-1, P-selectin and von Willebrand factor (Mills et al., 2007).

**Menopausal Status and Inflammation in Non-Breast Cancer Studies**

Although biomarkers provide important information about inflammation in breast cancer, the data are inconsistent. One possible explanation for the data variability is that breast cancer studies have not controlled for menopausal status.
Menopausal status can be divided into three categories: pre-menopausal, peri-menopausal and post-menopausal. Pre-menopausal refers to women having regular menstruations (Ringa, 2000). Peri-menopausal refers to women having irregular menstruations. Post-menopausal refers to women who have ceased to menstruate after a year of amenorrhea. The link between inflammation and menopause can be seen in the estrogen levels. Changes in estrogen levels that are associated with changes in the immune system include the menstrual cycle, menopause, aging, pregnancy, the use of oral contraceptives, hormonal replacement therapy and corticosteroids (Gameiro et al., 2010). Post-menopausal women have been found to undergo changes in the immune system due to age and estrogen deprivation (Gameiro et al., 2010).

*Menopausal Status and Breast Cancer Studies*

There have been a number of non-inflammatory biomarker breast cancer studies that have taken menopausal status into account when conducting research. In examining breast cancer patients after mastectomy and assigning them to different adjuvant chemotherapy groups, menopausal status was considered along with other factors (Caprini et al., 1980). In looking at adjuvant treatments which minimize the risk of relapse and death in breast cancer, it was found that only post-menopausal women benefited from aromatase inhibitors (Kelleher and Miles, 2003). Upon examining whether there are differences between women with breast cancer experiencing acute menopause symptoms and healthy women experiencing natural menopause, it was found that women receiving adjuvant systemic treatment were more likely to experience menopause symptoms than the healthy controls (McPhail...
and Smith, 2000). There was also a higher severity and incidence of specific menopause symptoms in women with breast cancer compared to controls. One study found that post-menopausal women had expressed statistically significant lower rates of progesterone in secondary tumors (Balleine et al., 1999). In both pre-menopausal and post-menopausal women, expression of estrogen in secondary tumors was low. In examining non-menopausal patients below 35 years old, non-menopausal patients above 35 years old and post-menopausal patients under 70 years old, it was determined that those below 35 years had significantly poorer metastasis free survival and overall survival (Bonnier et al., 1995).

One study examined the link between menopausal status and obesity in breast cancer patients (Chang et al., 2000). Although inflammation was not specifically examined, these patients already had high levels of inflammation since all of them were at a severe stage of inflammatory breast cancer. In examining the breast cancer patients who were divided into the pre or post-menopausal group, it was found that the post-menopausal group did better than the pre-menopausal group in terms of survival distribution (Chang, et al., 2000).

**Summary**

Studies examining the effects of breast cancer and its treatment on circulating markers of inflammation report inconsistent data. One reason for this inconsistency could be that prior studies have not taken into account the menopausal status of the study participants. This study therefore examined whether menopausal status is
associated with inflammation variability in breast cancer patients. My primary hypothesis was that pre-menopausal women with breast cancer, as compared to post-menopausal women with breast cancer, would have higher levels of inflammation after cycle 4 of chemotherapy compared to before chemotherapy. I also hypothesized that post-menopausal women with breast cancer would not have a significant change in their inflammatory markers compared to pre-menopausal women before and after chemotherapy.
Methods

Patients

Data from 85 women diagnosed with stage I-III breast cancer and referred for at least 4 cycles of adjuvant or neo-adjuvant anthracycline-based chemotherapy were studied. These participants were from two different University of California, San Diego (UCSD) breast cancer studies which followed the same chemotherapy regimen protocol and obtained the same inflammation, menopausal and psychosocial variables. One was the UCSD Fatigue, Sleep and Circadian Rhythms Study (Ancoli-Israel, Principal Investigator, NCI CA85264), which examined the relationship between sleep and fatigue before and during chemotherapy, and the other was the UCSD Contributions of Sleep/Rhythms/Fatigue to Chemobrain Study (Ancoli-Israel, Principal Investigator, NCI CA112035) which examined whether poor sleep and fatigue contribute to cognitive impairment during chemotherapy. Potential participants were recruited from the Rebecca and John Moores UCSD Cancer Center and from the San Diego community. Exclusion criteria included women undergoing bone marrow transplants, metastatic breast cancer, confounding underlying medical illnesses such as renal failure or significant preexisting anemia or other psychological or physical impairments that would limit participation. The study was approved by the UCSD Human Research Protection Program.

In the first study, data were collected before chemotherapy and during weeks 1, 2, and 3 of cycle 1 and cycle 4. In the second study, data were collected before
chemotherapy and at week 3 of cycle 4. Each chemotherapy cycle lasted 3 weeks.

The common data from each study, namely data collected before chemotherapy and at week 3 of cycle 4, were used for this thesis. The total time that elapsed between the first and last inflammatory, menopausal and psychosocial measurements was approximately 2.5 months.

Procedure

Blood samples for assessment of inflammatory markers, as well as menopausal status and menopausal symptoms, were obtained on two separate occasions: within a few days before starting chemotherapy, and again at week 3 of cycle 4 of chemotherapy. In addition to these measures, tumor estrogen and progesterone receptor status and several psychosocial assessments (namely depression, fatigue and quality of life) were collected at these same time points. The former data were collected to determine whether tumor receptor statuses serve as covariates. Women with breast cancer who are either estrogen or progesterone receptor positive have statistically favorable disease free, post recurrence and overall survival curves compared to those who are receptor negative (Kurebayashi et al., 1990). Furthermore, those who are both estrogen and progesterone receptor positive (ER+/PR+) are more sensitive to tamoxifen treatment compared to those who are estrogen receptor positive and progesterone receptor negative (ER+/PR-) (Arpino et al., 2005). The latter data were collected in order to replicate the literature and examine whether these psychosocial assessments serve as covariates.
Inflammatory markers

The inflammatory markers that were examined are VEGF (vascular endothelial growth factor), IL-6 (interleukin 6), sICAM-1 (the soluble form of ICAM-1), P-selectin, CRP (C-reactive protein) and TNF-alpha (tumor necrosis factor alpha). VEGF is an endothelial mitogen which causes the formation, migration and proliferation of new blood vessels required for tumor growth and metastasis in breast cancer (Boudreau and Myers, 2003). IL-6 is a cytokine produced by macrophages, endothelial cells, B cells, T cells and tumor cells (Salgado et al., 2003). IL-6 has been shown to be a moderator of multi-drug resistance (Conze et al., 2001). sICAM-1 is indicative of the acute phase response (Blann et al, 2002) and is significantly higher in breast cancer patients compared to controls (Klein et al., 1995). P-selectin is indicative of elevated platelet activation in breast cancer (Blann et al., 2001). CRP is synthesized by the liver in response to inflammation (Mills et al., 2007) while the cytokine TNF-alpha predicts progression free survival in metastatic breast cancer patients (Bozcuk et al., 2004).

Whole blood was preserved with EDTA. Following centrifugation, the plasma was stored at -80º Celsius until assay. The levels of the inflammatory markers sICAM-1, VEGF, IL-6, CRP, TNF-alpha and P-selectin were determined by commercial ELISA kits (R&D Systems, Mpls., MN) (Mills et al., 2002, 2003). The intra-assay coefficients of variation for all of the ELISA assays were less than 5%. The inter-assay coefficients of variation for all of the ELISA assays were less than...
10%. See Appendix for the standard curve plots for each of the biomarker ELISA assays.

**Menopausal Status and Symptoms**

Menopausal status and symptoms were assessed with the Greene Climacteric Scale and with 4 additional questions about regular menses before/after chemotherapy (pre-menopausal status), irregular menses before/after chemotherapy (peri-menopausal status), post-menstrual before/after chemotherapy (post-menopausal status) and post-hysterectomy or post-oophorectomy before/after chemotherapy.

The Greene Climacteric Scale is a self-report questionnaire which determines the presence and severity of a wide range of menopausal symptoms (Greene, 1998). There are six separate subscales measuring somatic (headaches, muscle and joint pains), vasomotor (hot flashes, night sweats), sex, anxiety, depression and psychological (the sum of anxiety and depression). The sum of the scores from each subscale generates a total Greene Climacteric Scale score. The score can range from 0 to 66. A higher score reflects a more serious menopausal problem. The Greene Climacteric Scale has a predictive validity which has been established in several studies (Pearce et al., 1997; Wu et al., 2001).

**Psychosocial Questionnaires**

Previous studies have shown that psychosocial variables like depression, fatigue and quality of life are related to changes in inflammation in women with breast
cancer (Mills et al., 2004). To replicate the literature, these psychosocial measures were also taken into account when analyzing the data.

Depression was determined using the Center of Epidemiological Studies-Depression (CES-D) (Radloff, 1977). The CES-D is self-reported questionnaire that consists of a 20 item scale of depressive symptoms. The total score can range from 0 to 60 with higher scores indicating greater distress. The scale has been shown to have high validity and reliability in the assessment of depressive symptoms. Since the CES-D reflects cognitive and affective symptoms instead of somatic symptoms of depression, it is highly advised to use for patients who have medical problems.

Fatigue was assessed using the short form of the Multidimensional Fatigue Symptom Inventory (MFSI-SF) (Stein et al., 1998). The original MFSI was an 83 item self-reported questionnaire designed to assess the principle manifestations of fatigue. The total score can range from -24 to 96 with higher scores indicating more fatigue. The MFSI is a valid and reliable tool to evaluate fatigue in breast cancer patients (Stein et al, 2004).

Quality of life was determined by the Functional Assessment of Cancer Therapy-Breast (FACT-B) (Cella, 1996). There are five separate subscales measuring physical well-being, social well-being, emotional well-being, functional well-being and breast cancer symptoms. The sum of the scores from each subscale generates a FACT-B total score. The total score can range from 0 to 144 with higher scores
indicating a better quality of life. The FACT-B has shown reliability and validity in cancer populations (Yellen et al., 1997; Brady et al., 1997).

Data analysis

Subjects were divided into two groups according to menopausal status using the information from the questions that asked about menopausal status pre and post chemotherapy. There were 29 women who were either pre-menopausal or peri-menopausal before chemotherapy and pre-menopausal or peri-menopausal after chemotherapy (pre/peri) and 56 women who were peri-menopausal or post-menopausal before chemotherapy and post-menopausal after chemotherapy (peri/post). Pre-menopausal status was defined as regular menstruations, peri-menopausal was defined as irregular menstruations and post-menopausal status was defined as no menstruations or post-hysterectomy. The Greene Climacteric scale was used to determine the presence and severity of menopausal symptoms.

Independent sample T-tests, repeated measures analysis of variance, Spearman correlations, multiple regressions and canonical correlations were used to determine whether menopausal status has an effect on inflammatory markers (SPSS Statistical Software, Chicago, IL) prior to chemotherapy as well as at week 3 of cycle 4 of chemotherapy.

More specifically, separate multiple regression analyses were conducted to see if menopausal status predicted the inflammatory response to chemotherapy. The respective post-chemotherapy inflammatory marker was the dependent variable, and
age, BMI, the respective pre-chemotherapy inflammatory marker, and menopausal status (pre/peri and peri/post) were the independent predictor variables. If a non-Gaussian distribution of an inflammatory marker was found, that inflammatory marker was log transformed.

Canonical correlation tests were performed to determine if there were any relationships between the set of inflammatory markers and the set of Greene Climacteric sub-scores or total scores. This is a parsimonious method for finding maximum correlations between sets of multivariate data and therefore has better statistical power than bivariate correlations between each pair of variables. The following canonical correlation tests were conducted: 1) between the inflammatory markers before chemotherapy and the Greene Climacteric sub-scores before chemotherapy 2) the inflammatory markers before chemotherapy and the Greene Climacteric total scores before chemotherapy 3) the inflammatory markers after chemotherapy and the Greene Climacteric sub-scores after chemotherapy and 4) the inflammatory markers after chemotherapy and the Greene Climacteric total scores after chemotherapy.

Chi-square tests and independent sample T-tests were conducted to determine if there were any covariates. Chi-square tests were conducted on menopausal status and the following variables: stage of disease, treatment, tumor estrogen receptor status, tumor progesterone receptor status (tumor receptor status was obtained on only a subset of 47 study participants), ethnicity, education, marital status, occupation and income. With the exception of stage of disease and treatment, all the other variables
mentioned above were divided into two groups. Both tumor estrogen receptor status and tumor progesterone receptor status were divided into positive and negative statuses. Ethnicity was divided into Caucasian and other. Education was divided into college graduate and other. Marital status was divided into married and other. Occupation was divided into professional and other.

There were several different chemotherapy treatment regimens that the study participants had undergone. These different regiments were collapsed into three primary treatment groups. The first group consisted of exactly 4 cycles of anthracycline chemotherapy, more than 4 cycles of anthracycline chemotherapy and 4 or more cycles of anthracycline chemotherapy that had taken concurrently 5 Fluorouracil. The second group consisted of exactly 4 cycles of anthracycline and Taxotere, exactly 4 cycles of anthracycline and Taxol, exactly 4 cycles of anthracycline followed by Taxotere, and exactly 4 cycles of anthracycline followed by Taxol. The third group had the other treatments which consisted of exactly 4 cycles of Adriamycin and Cyclophosphamid, 4 or more cycles of Epirubicin and Cytoxan taken concurrently, and 4 or more cycles of Adriamycin concurrent with Taxol.

The psychosocial data MFSI, CES-D and FACT-B were examined by independent sample T-tests by menopausal status.

For all analyses, P values less than 0.05 were considered statistically significant.
Results

Sociodemographic Characteristics

Although the menopausal groups were significantly different in mean age (P < 0.01), they were similar in BMI, cancer stage, ethnicity, marital status, education, income and occupation (see Table 1). The groups were also similar in treatment (see Table 2), estrogen receptor status and progesterone receptor status (see Table 3). At p = 0.069, the estrogen status is fairly close to statistical significance with the post-menopausal women having more estrogen receptors (ER+).

Inflammatory Characteristics

There were no significant differences between the two menopausal groups on any of the inflammatory markers. The mean concentration and standard error (SE) for each inflammatory marker is grouped by menopausal status before chemotherapy and after chemotherapy (see Figures 1-6). In addition to presenting data for the study participants by menopause group, for each biomarker data is presented for non-breast cancer control participants, N = 12. These control women, who were age-matched for the patients, were recruited as part of the parent studies and had data collected at the same time points (i.e. before the patients started chemotherapy and 2.5 months later). Menopausal status is not available for these control participants, and therefore formal statistical group comparison tests of the biomarkers between the controls and the menopausal groups of the breast cancer patients were not conducted.
Psychosocial Characteristics

There were no significant differences between the two menopausal groups in MFSI, CES-D or FACT-B before chemotherapy or after chemotherapy (see Table 4).

Effects of Chemotherapy on Inflammation

Independent sample T-tests by menopausal group which were conducted on each inflammatory marker before chemotherapy and at week 3 of cycle 4 of chemotherapy showed no significant differences ($P > 0.05$). Independent sample T-tests were also conducted on the change scores (pre minus post chemotherapy) of each inflammatory marker which also showed no significant differences ($P > 0.05$).

Spearman correlations were also conducted using the Greene Climacteric Scale total score as a continuous variable. In examining the correlations between inflammatory markers and the menopausal symptoms before chemotherapy and after chemotherapy it was determined that there were no statistically significant relationships. In examining the correlations between the change scores for each inflammatory marker and menopausal symptoms as determined by the Greene Climacteric score before chemotherapy and after chemotherapy, no relationship was found.

Multiple regression analyses showed that menopausal status, BMI and age did not predict the post-chemotherapy inflammatory level (see Table 5). The respective pre-chemotherapy inflammatory markers did predict the post-chemotherapy level for each biomarker with the exception of P-selectin.
Canonical correlation tests showed no relationships between the inflammatory markers and the Greene Climacteric sub-scores or total scores.

*Effects of Chemotherapy on Psychosocial Characteristics*

In order to replicate the literature and look for effects of covariates, independent sample T-tests by menopausal group were conducted on MFSI, CES-D and FACT-B. There were no significant differences (P > 0.05).
Discussion

**Summary of Results**

This study examined the relationship between inflammatory markers and menopausal status in women with breast cancer. Outcomes of interest were examined prior to the start of chemotherapy and about 2.5 months later after the third week of cycle 4 of chemotherapy. The results from the statistical analysis show that menopausal status does not account for biomarker variability in this population. The study included analysis of a number of inflammatory biomarkers that are of relevance to breast cancer and its clinical course, and none were found to be influenced by menopausal status. A prior study in which the inflammatory biomarker IL-6 was examined as a predictor of survival in patients with metastatic breast cancer was not found to be correlated with menopausal status (Salgado et al., 2002).

The study also examined several relevant psychosocial characteristics. This was done to replicate the literature and determine whether these characteristics were covariates. The findings were that unlike previous studies, there were no significant differences between the two menopausal groups in MFSI, CES-D or FACT-B before chemotherapy or after chemotherapy, which means that these characteristics were not covariates for this particular study.
Study Limitations

There are several limitations in this study. The drawing of the blood did not occur at the same time of day, which could have had a bearing on the levels of some of the biomarkers, including IL-6 and TNF-alpha, which have evidence of circadian rhythms. Although all blood samples were kept on ice, some were not delivered to the research lab for processing on the same day the blood was drawn. There were a limited number of patients who had tumor estrogen and progesterone receptor status data available, thus limiting the statistical power for those particular analyses. Another limitation is that menopausal status was defined according to women’s self-reports and not objectively. Future studies should consider examining menopausal status based on Follicle Stimulating Hormone (FSH) levels in combination with women’s self-reports.

Conclusion

This study found no relationship between menopausal status and inflammatory markers in women with breast cancer undergoing chemotherapy treatment, suggesting that other factor(s) are responsible for the amount of variation and inconsistency of inflammatory biomarkers seen in the literature. If the findings from this study were to hold true in future research, an implication is that researchers and physicians do not need to take the menopausal status of women with breast cancer into account when considering potential inflammatory responses to standard treatment to breast cancer.
Figure 1: CRP Mean Concentration in age-matched non-breast cancer control women and in breast cancer study participants according to menopausal status pre and post chemotherapy. The control group, N = 12, is not divided by menopausal status. For Pre/Peri, N = 29 and for Peri/Post, N = 56. Pre = pre chemotherapy. C4W3 = Cycle 4 Week 3 = post chemotherapy.
Figure 2: sICAM-1 Mean Concentration in age-matched non-breast cancer control women and in breast cancer study participants according to menopausal status pre and post chemotherapy. The control group, N = 12, is not divided by menopausal status. For Pre/Peri, N = 29 and for Peri/Post, N = 56. Pre = pre chemotherapy. C4W3 = Cycle 4 Week 3 = post chemotherapy.
Figure 3: IL-6 Mean Concentration in age-matched non-breast cancer control women and in breast cancer study participants according to menopausal status pre and post chemotherapy. The control group, N = 12, is not divided by menopausal status. For Pre/Peri, N = 29 and for Peri/Post, N = 56. Pre = pre chemotherapy. C4W3 = Cycle 4 Week 3 = post chemotherapy.
Figure 4: TNF-alpha Mean Concentration in age-matched non-breast cancer control women and in breast cancer study participants according to menopausal status pre and post chemotherapy. The control group, N = 12, is not divided by menopausal status. For Pre/Peri, N = 29 and for Peri/Post, N = 56. Pre = pre chemotherapy. C4W3 = Cycle 4 Week 3 = post chemotherapy.
Figure 5: P-selectin Mean Concentration in age-matched non-breast cancer control women and in breast cancer study participants according to menopausal status pre and post chemotherapy. The control group, N = 12, is not divided by menopausal status. For Pre/Peri, N = 29 and for Peri/Post, N = 56. Pre = pre chemotherapy. C4W3 = Cycle 4 Week 3 = post chemotherapy.
Figure 6: VEGF Mean Concentration in age-matched non-breast cancer control women and in breast cancer study participants according to menopausal status pre and post chemotherapy. The control group, N = 12, is not divided by menopausal status. For Pre/Peri, N = 29 and for Peri/Post, N = 56. Pre = pre chemotherapy. C4W3 = Cycle 4 Week 3 = post chemotherapy.
Tables

Table 1: Demographics by Menopausal Status
<table>
<thead>
<tr>
<th>Group</th>
<th>Pre/Peri, N = 29</th>
<th>Peri/Post, N = 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, years (SD)</td>
<td>43.31 (6.21)</td>
<td>55.91 (6.96)</td>
</tr>
<tr>
<td>Mean BMI (SD)</td>
<td>27.40 (6.45)</td>
<td>27.91 (5.84)</td>
</tr>
<tr>
<td>Stage of disease, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>7 (24.14)</td>
<td>13 (23.21)</td>
</tr>
<tr>
<td>II</td>
<td>14 (48.28)</td>
<td>19 (33.93)</td>
</tr>
<tr>
<td>III</td>
<td>4 (13.79)</td>
<td>8 (14.29)</td>
</tr>
<tr>
<td>III-A</td>
<td>2 (6.90)</td>
<td>7 (12.50)</td>
</tr>
<tr>
<td>Ethnicity, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>20 (68.97)</td>
<td>48 (85.71)</td>
</tr>
<tr>
<td>African American</td>
<td>1 (3.45)</td>
<td>3 (5.36)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1 (3.45)</td>
<td>2 (3.57)</td>
</tr>
<tr>
<td>Asian</td>
<td>3 (10.34)</td>
<td>3 (5.36)</td>
</tr>
<tr>
<td>Native American</td>
<td>2 (6.90)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Mixed</td>
<td>1 (3.45)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (3.45)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Mean education, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never attended school</td>
<td>1 (3.45)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Completed primary school</td>
<td>0 (0.00)</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>Some high school</td>
<td>0 (0.00)</td>
<td>1 (1.79)</td>
</tr>
<tr>
<td>Completed high school</td>
<td>5 (17.24)</td>
<td>6 (10.71)</td>
</tr>
<tr>
<td>Some college</td>
<td>6 (20.69)</td>
<td>22 (39.29)</td>
</tr>
<tr>
<td>Completed college</td>
<td>17 (58.62)</td>
<td>27 (48.21)</td>
</tr>
<tr>
<td>Income, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than $30,000</td>
<td>1 (3.45)</td>
<td>9 (16.07)</td>
</tr>
<tr>
<td>Greater than $30,000</td>
<td>24 (82.76)</td>
<td>39 (69.64)</td>
</tr>
<tr>
<td>Unknown</td>
<td>1 (3.45)</td>
<td>1 (1.79)</td>
</tr>
<tr>
<td>Refused to answer</td>
<td>3 (10.34)</td>
<td>7 (12.5)</td>
</tr>
<tr>
<td>Marital status, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never married</td>
<td>4 (13.79)</td>
<td>4 (7.14)</td>
</tr>
<tr>
<td>Divorced</td>
<td>3 (10.34)</td>
<td>15 (26.79)</td>
</tr>
<tr>
<td>Separated</td>
<td>1 (3.45)</td>
<td>2 (3.57)</td>
</tr>
<tr>
<td>Widowed</td>
<td>0 (0.00)</td>
<td>4 (7.14)</td>
</tr>
<tr>
<td>Married</td>
<td>21 (72.41)</td>
<td>31 (55.36)</td>
</tr>
<tr>
<td>Mean occupation, N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Professional</td>
<td>19 (65.51)</td>
<td>32 (51.14)</td>
</tr>
<tr>
<td>Clerical</td>
<td>1 (3.45)</td>
<td>4 (7.14)</td>
</tr>
<tr>
<td>Trade/Service</td>
<td>3 (10.34)</td>
<td>3 (5.36)</td>
</tr>
<tr>
<td>Homemaker</td>
<td>3 (10.34)</td>
<td>6 (10.71)</td>
</tr>
<tr>
<td>Retired</td>
<td>0 (0.00)</td>
<td>4 (7.14)</td>
</tr>
</tbody>
</table>
Table 2: Treatment by Menopausal Status

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre/Peri, N = 29</th>
<th>Peri/Post, N = 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
<td>24</td>
</tr>
</tbody>
</table>

P = 0.219.

**Group 1** = exactly 4 cycles of anthracycline chemotherapy, more than 4 cycles of anthracycline chemotherapy and 4 or more cycles of anthracycline chemotherapy that was taken concurrently with 5 Fluorourac.

**Group 2** = exactly 4 cycles of anthracycline and Taxotere, exactly 4 cycles of anthracycline and Taxol, exactly 4 cycles of anthracycline followed by Taxotere and exactly 4 cycles of anthracycline followed by Taxol.

**Group 3** = all other treatments including 4 cycles of Adriamycin and Cyclophosphamid, 4 or more cycles of Epirubicin and Cytoxan that were taken concurrently and 4 or more cycles of Adriamycin that was taken concurrently with Taxol.
Table 3: Tumor Estrogen and Progesterone Receptor Status by Menopausal Status

<table>
<thead>
<tr>
<th>Receptor Status</th>
<th>Estrogen</th>
<th>Progesterone</th>
<th>Estrogen</th>
<th>Progesterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Positive</td>
<td>7</td>
<td>7</td>
<td>22</td>
<td>16</td>
</tr>
</tbody>
</table>

Tumor estrogen and progesterone receptor status obtained on only a subset of study participants, N = 47.
For estrogen, p = 0.069.
For progesterone, p = 0.609.
Table 4: Mean (SD) for Psychosocial Characteristics pre and post chemotherapy

<table>
<thead>
<tr>
<th>Psychosocial</th>
<th>Pre/Peri, N = 29</th>
<th>Peri/Post, N = 56</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>C4W3</td>
</tr>
<tr>
<td>MFSI-SF Fatigue</td>
<td>6.57 (15.49)</td>
<td>17.05 (21.73)</td>
</tr>
<tr>
<td>CES-D Depression</td>
<td>10.04 (6.36)</td>
<td>16.31 (10.41)</td>
</tr>
<tr>
<td>FACT-B Quality of Life</td>
<td>105.22 (13.94)</td>
<td>96.95 (18.94)</td>
</tr>
</tbody>
</table>

Pre = pre chemotherapy.
C4W3 = Cycle 4 Week 3 = post chemotherapy.
Table 5: Regression to predict Inflammatory Markers post chemotherapy

<table>
<thead>
<tr>
<th>Inflammatory Marker at C4W3</th>
<th>Pre/Peri, N = 29 and Peri/Post, N = 56</th>
<th>Respective Pre-Chemotherapy Inflammatory Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>B = 0.05, p = 0.67</td>
<td>B = 0.57, p = 0.02</td>
</tr>
<tr>
<td>sICAM-1</td>
<td>B = 0.00, p = 0.79</td>
<td>B = 0.88, p = 0.00</td>
</tr>
<tr>
<td>IL-6</td>
<td>B = 0.07, p = 0.22</td>
<td>B = 0.63, p = 0.00</td>
</tr>
<tr>
<td>TNF-alpha</td>
<td>B = 0.08, p = 0.07</td>
<td>B = 0.72, p = 0.00</td>
</tr>
<tr>
<td>P-selectin</td>
<td>B = 0.00, p = 0.93</td>
<td>B = 0.16, p = 0.35</td>
</tr>
<tr>
<td>VEGF</td>
<td>B = 0.11, p = 0.25</td>
<td>B = 0.31, p = 0.04</td>
</tr>
</tbody>
</table>

* Adjusted for age and BMI.
C4W3 = Cycle 4 Week 3 = post chemotherapy.
Appendix: Presentation of the ELISA assay standard curves for each of the inflammatory biomarkers measured.

Standard Curve for CRP
Standard Curve for sICAM-1

Signal vs. Concentration (ng/mL)

0  50  100  150
0  150000  300000  450000  600000
Standard Curve for IL-6
Standard Curve for TNF-alpha
Standard Curve for P-selectin
Standard Curve for VEGF

Average Optical Density

Concentration (pg/mL)
References


