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Depressive Symptomology, Pro-Inflammatory Cytokines, and S100A8/A9 Heterodimer Levels in Post-Surgical Non-Metastatic Breast Cancer Patients: Test of Direct and Indirect Effect

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DEPRESSIVE SYMPTOMOLOGY, PRO-INFLAMMATORY CYTOKINES, AND
S100A8/A9 HETERODIMER LEVELS IN POST-SURGICAL NON- METASTATIC
BREAST CANCER PATIENTS: TEST OF DIRECT AND INDIRECT EFFECT

By

Erica R. Nahin

A THESIS

Submitted to the faculty
of the University of Miami
in partial fulfillment of the requirements
for the degree of Master of Science

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Depressive Symptomology, Pro-Inflammatory Cytokines,
and S100A8/A9 Heterodimer Levels in Post-Surgical
Non-Metastatic Breast Cancer Patients: Test of Direct and Indirect
Effect

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The link between depression and breast cancer outcomes has been a topic of great interest for the last decade and up-regulated inflammatory signaling may be one of the biological mechanisms at play. The purpose of this study was to examine the relationship between depression severity levels, and pro-inflammatory cytokines interleukin – 1 beta (IL-1 β), IL-6, and tumor necrosis factor-alpha (TNF- α) in tandem with a pathway involving the receptor for advanced glycation end products (RAGE) 2-10 weeks post-surgery, and 1 year later. Activation of RAGE by ligands S100A8/A9 can trigger pathways that lead to the production of pro-inflammatory cytokines. To date, no studies have linked depression with RAGE ligands nor tested whether these S100A8/A9 ligands act as an intermediary between depressive symptoms and pro-inflammatory cytokine up-regulation in breast cancer patients.

Women in the South Florida area (ages 32-69) with Stage 0-III breast cancer (N = 240) were recruited 2 - 10 weeks post-surgery between December 1998 and February 2005 as part of a randomized controlled trial of a stress management intervention. They were interviewed with the Hamilton Rating Scale for Depression (HRSD) and provided blood

samples before initiating adjuvant chemotherapy or radiation. Participants in the sample for this thesis were those who had cryopreserved blood samples available for analysis of immune parameters (N= 59). They were predominately non-Hispanic white (72.9%), followed by Hispanic (20.3%) and Black (5.1%).

Using linear regression, it was found that after controlling for age, stage, body mass index (BMI), time since surgery, and type of surgery (mastectomy versus lumpectomy), depression severity related to significantly greater levels of S100A8/A9 ($p = .038$), IL-1 β ($p = .011$) and TNF- α ($p = .026$), and marginally with IL-6 levels ($p = .062$) at the post-surgical time point. Regression analyses indicated that S100A8/A9 served as an intermediary in the relationship between depression and circulating cytokines, demonstrating an indirect effect for IL-1 β (effect = .058, 95% CI [.0009, .1687]) and IL-6 (effect = .064, 95% CI [.0070, .1685]) but not for TNF- α at the post-surgical time point. There was no significant relationship of pro-inflammatory cytokines or s100A8/A9 levels with depression at the one-year follow-up. An exploratory analysis indicated a similar pattern of results using affective and neurovegetative depressive symptom clusters at the post-surgical time point. Analyses revealed that depressive symptom clusters were not associated with S100A8/A9, IL-1 β , IL-6 or TNF- α at the 12 month follow-up.

These data suggest that depression may relate to greater inflammation in post-surgical breast cancer patients, and that the association between depression and pro-inflammatory cytokine levels may operate indirectly via activation of RAGE. The absence of these relationships at the 12-month follow-up suggests that other factors (e.g., recent adjuvant therapy regimen) may obscure associations between mood and inflammatory markers at this point in time. Because inflammatory processes may increase odds of metastasis,

addressing depression early in the post-surgical period may have future health effects in breast cancer patients. However, the absence of these associations at the 12-month point suggests that these results need to be replicated in an independent sample.

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CHAPTER 1: INTRODUCTION

In 2013, about 230,000 women were diagnosed with breast cancer in the U.S. Of those about, 41,000 women died from their cancer (National Cancer Institute, 2016). It is common for breast cancer patients to feel some level of depressive symptomology with a breast cancer diagnosis (Bower, 2008). In a 2010 meta-analysis by Pinquart and Duberstein it was found that cancer patients with depression both before and after the cancer diagnosis had significantly increased rates of mortality independent of cancer stage. In another study, patients with breast cancer who had a depression diagnosis less than two years prior to their cancer diagnosis were found to have significantly decreased chances of survival (Goodwin et al., 2004). More specifically, breast cancer patients who score highly on tests of helplessness and hopelessness tend to have shorter relapse-free survival within 5 years of diagnosis (Watson, 1999). Additionally, post-operative depression in breast cancer patients has been found to be a significant predictor of all-cause mortality (Hjerl et al., 2003). Since the link between depression and poorer breast cancer prognosis has been established, it is now important to understand the biological mechanisms at play that may account for the association between depressive symptoms and disease outcomes. This may allow medical professionals additional treatment options to maximize the chances of a positive disease outcome.

Breast Cancer Pathology & Treatment

Breast cancer is a complex, genetic disease, consisting of a number of subtypes with differing prognoses due to a number of molecular alterations (Lal, McCart Reed, de Luca, & Simpson, 2017; Rakha, Reis-Filho, & Ellis, 2010). Tumor stage is thought to be

the most important determinant of disease outcome in women with breast cancer, while in non-metastatic patients axillary spread is the most important determinant of recurrence (Warner, 2011). Regular breast cancer screening via mammography helps detect cancer before the tumor becomes palpable (Warner, 2011). To correctly diagnose breast cancer, a pathologist collects and examines a tissue biopsy and resection specimen (Lal et al., 2017). Using this information, they are able to determine histological type, tumor grade, and tumor stage (Lal et al., 2017). The most common breast cancer subtype, consisting of 80% of diagnoses, is Invasive Carcinoma of No Special Type (previously called Invasive Ductal Carcinoma) (Lal et al., 2017). There are a total of 20 different histological subtypes, and the remaining are considered “special” histological types due to their unique growth patterns (Lal et al., 2017). The most common special type, consisting of 5-15% of diagnoses, is Invasive Lobular Carcinoma (Lal et al., 2017).

Accurate diagnosis of the cancer type is vital as each part of the diagnostic system describes the way that the tumor behaves and grows (Lal et al., 2017). Histological grade describes tumor cell differentiation, or the abnormality of the way the tumor looks relative to normal tissue (Lal et al., 2017). A histological grade 1 tumor is significantly more differentiated than a histological grade 3 tumor and also has better prognosis compared with a grade 3 tumor (Lal et al., 2017). Tumor stage measures how far the tumor has spread in the body and has a strong indicator of prognosis (Lal et al., 2017). The American Joint Committee on Cancer TNM staging system is used for breast cancer in the same way that it is used for cancers of other organs (Lal et al., 2017). T represents the tumor size and whether or not the tumor has invaded the chest wall, N represents the number of lymph nodes with cancer present, and M represents distant metastasis (Lal et

al., 2017). If distant metastasis is present then there is cancer present in parts of the body besides the location of the primary tumor (Lal et al., 2017).

In addition to characterizing breast cancer based on histological type and stage, there are also a number of biomarkers which have prognostic value and help determine appropriate treatment. Some of the most important biomarkers are the Oestrogen Receptor (ER), the Progesterone Receptor (PR) and the Human Epidermal Growth factor Receptor 2 (HER2/ERBB2) (Lal et al., 2017). ER, PR, and HER2 protein levels are assessed using immunohistochemistry while ERBB2 gene copy number is assessed using in situ hybridization (Rakha et al., 2010). If a person has ER and/or PR receptors, then their cancer is considered hormone receptor positive (HR+) (Lal et al., 2017). The vast majority of cancers are HR+, with 60-75% of cancers being estrogen receptor-positive (ER+) of which 65% are progesterone receptor-positive (PgR+) (Burstein et al., 2014). When a person's cancer is ER- and PgR+ adjuvant endocrine therapy is highly effective in treating the cancer (Burstein et al., 2014). When a tumor is negative for ER, PR, and HER2, it is considered to be a "triple-negative" breast cancer (Lal et al., 2017). Although these tumors are responsive to chemotherapy, the outcome is poor compared with other types of tumors (Lal et al., 2017). Another important biomarker is Ki67. Ki67 is a marker commonly used to predict the rate of growth of the tumor (Lal et al., 2017). Together, ER, PR, HER2, and Ki67 comprise a tumor's protein-based signature, called the IHC4 (Lal et al., 2017).

Based on the subtype of cancer and the protein-based signature, a treatment provider is able to determine the best course of treatment. When a cancer is considered ductal carcinoma in situ (DCIS) or in the early stages, many women are recommended

surgical intervention to remove the tumor (American Society of Clinical Oncology (ASCO), 2017). During the surgery, the surgeon removes the tumor as well as a margin of healthy tissue to ensure the entire tumor is removed (ASCO, 2017). During the surgery, the axillary lymph nodes are also examined for evidence of cancer. Sometimes a sentinel lymph node biopsy is performed to determine if cancer has spread (ASCO, 2017). The lymph nodes examined during this procedure receive lymph drainage from the breast (ASCO, 2017). If cancer is present in the lymph nodes, an axillary lymph dissection is performed where many lymph nodes are removed (ASCO, 2017). If the tumor is larger or growing quickly, women are often recommended chemotherapy, radiation therapy, or hormone therapy prior to surgery in order to shrink the tumor, called neoadjuvant therapy (ASCO, 2017). The benefits of completing neoadjuvant therapy depend on the case, however it often makes the surgery easier to perform and some women are able to have a lumpectomy instead of mastectomy, thus preserving the breast (ASCO, 2017). Adjuvant therapy is treatment received following surgical procedures. This often includes chemotherapy, radiation, and/or hormone therapy.

Breast Cancer Metastasis

Though the majority of breast cancers are diagnosed prior to metastasis, approximately 20-30% of women diagnosed with only primary tumors develop metastasis, most commonly to the bone (Kennecke, 2010). Most deaths attributed to breast cancer are due to metastasis and not the primary tumor (Weigelt, Peterse, & van't Veer, 2010). In 2012, Scully, Bay, Yip, & Yu published a review article examining detection of metastasis, mechanisms involved with metastasis, and treatment. In terms of detecting metastasis, oftentimes medical professionals rely on identifying clinical

manifestations of the spread to distant organs (Scully et al., 2012). They also often biopsy the affected organ, complete radiological evaluations and other imaging methods, and use serum tumor markers like circulating tumor cells (CTC's) (Lucroix, 2011; Sun et al., 2011).

In order for breast cancer to metastasize, there are a number of steps that need to be completed sequentially (Scully et al., 2012). First, cells from the primary tumor invade the surrounding host tissue until they invade and intravasate the blood or lymphatic tissue (Hunter, Crawford & Alsarraj, 2008). When the tumor cells invade local host tissue, cell to cell adhesion and cell adhesion to the extracellular matrix are disrupted (Li & Feng, 2011). Next the tumor cells move through the blood stream to distant organs (Hunter, Crawford & Alsarraj, 2008). At this time, the tumor cell's cell cycle arrests and they adhere to the capillary beds in the organ (Hunter, Crawford & Alsarraj, 2008). They then extravasate into the parenchyma of the organ leading to proliferation and angiogenesis (Hunter, Crawford & Alsarraj, 2008). At the same time these steps take place, the tumor cell needs to evade the immune response and signals to the cell promoting apoptosis (Hunter, Crawford & Alsarraj, 2008).

The tumor microenvironment plays a crucial role in breast cancer metastasis. The microenvironment consists of immune cells, fibroblast cells, endothelial cells, and mural cells (Hunter, Crawford & Alasarraj, 2008). When the malignant cell interacts with cells in the microenvironment, metastasis becomes possible (Coghilin & Murray, 2010). For example, macrophages recruited from non-invasive breast cancer cells may induce angiogenesis and promote transformation of the non-invasive cells to malignant cells (Lin & Pollard, 2007). In the bone, interactions between osteoclasts, osteoblasts and tumor

cells lead to metastasis (Mundy, 2002; Parker & Sukumar, 2003). Other times, the tumor cells secrete substances which make metastasis possible. In 2002 Hiratsuki et al found that tumor cells' signal induced MMP9 gene expression in lung endothelial cells and macrophages before the onset of metastasis. This lead to tumor cell invasion in the lungs (Hiratsuki et al., 2002).

Breast Cancer Risk Factors

Breast cancer results from a combination of factors including a person's genes, environment, and behaviors (Lacey Jr. et al., 2009). In 2009 Lacey Jr. et al. published a study which examined risk factors of 2,085 breast cancer patients. This was part of a larger study involving 70,575 women who were randomized in a Prostate, Lung, Colorectal, and Ovarian Screening Trial (Lacey Jr. et al., 2009). Women included were also all post-menopausal (Lacey Jr. et al., 2009). They found that consistent with previous research, increasing age, having children, family history of breast cancer, and the use of hormone therapy during menopause were significant risk factors (Lacey Jr. et al., 2009).

Height and weight have also been a focus of much research on breast cancer risk (van den Brandt et al., 2000). This study found a positive association in post-menopausal women for both height and weight, but not for pre-menopausal women (van den Brandt et al., 2000). They found a significant inverse relationship for associations with risk for pre-menopausal women (van den Brandt et al., 2000). One of the explanations proposed in this article was that in post-menopausal women there might be increased estrogen levels near adipose tissue, thus increasing risk of tumor growth (van den Brandt et al., 2000, Ziegler et al., 1996). They also note the previous finding that there are higher levels

of estrone and estradiol in obese post-menopausal women compared with those of normal weight (van den Brandt et al., 2000, Hankinsen et al., 1995, Cauley et al., 1989). In 2003, the Endogenous Hormones Breast Cancer Collaborative Group (EHBCCG) published an article examining BMI, and sex hormone levels in post-menopausal breast cancer patients. Similar to previous literature, they found there was a positive association between BMI and breast cancer risk which was reduced when they controlled for serum estrogen concentrations (EHBCCG, 2003). They also found that there was a decreased risk when they adjusted for free estradiol, as well as other estrogens such as total estradiol and non-sex hormone-binding globulin-bound estradiol (EHBCCG, 2003). It was therefore concluded that increasing BMI is associated with increased breast cancer risk because of increase in estrogens (EHBCCG, 2003).

Another risk factor of breast cancer is diabetes. A meta-analysis published in 2007 by Larsson, Mantzoros, and Wolk showed that women with diabetes had a 20% increased risk of developing breast cancer. Some of the mechanisms proposed include alterations in the concentration of insulin, insulin-like growth factors, and endogenous sex hormones. In type II diabetes, there is usually insulin resistance and increased secretion of insulin from the pancreas for long periods of time before the onset of the disease as well as after (Larsson, Mantzoros, & Wolk, 2007). Previous research has shown that insulin has mitogenic effects on breast cancer tissue and insulin receptors are over-expressed in breast cancer cells (Larsson, Mantzoros, & Wolk, 2007; Chappell et al., 2001; Papa & Belfiore, 1996). In addition, high levels of fasting glucose as well as presence of type II diabetes has been found to be associated with negative prognostic markers and poor

disease outcome (Larsson, Mantzoros, & Wolk, 2007; Goodwin et al., 2002; Wolf et al., 2006).

In addition to these modifiable breast cancer risk factors, there is also evidence that modifiable psychosocial factors, such as depression, are associated with poorer clinical outcomes in diagnosed cases (Hjerl et al., 2003; Antoni et al., 2017). Depression has also been associated with greater levels of inflammatory biomarkers, raising the possibility that depression may relate to poorer disease outcomes by way of its association with inflammation. The next section summarizes research tying depression to inflammatory markers in women with breast cancer.

Breast Cancer, Depressive Symptomology, and Pro-inflammatory Cytokines

Most current literature estimates the prevalence of depressive symptoms within breast cancer patients to be between 20-30%, with 9% meeting criteria for major depressive disorder (MDD) (Bower, 2008). Furthermore, cancer patients with depression tend to have greater rates of cancer progression, incidence, and mortality than patients without depression (Spiegel & Giese-Davis, 2003). It is therefore imperative to understand biologically what is leading cancer patients with depressive symptoms to have worse disease outcomes than cancer patients without depressive symptoms. This is especially important for breast cancer patients due to the high rates of depression comorbidity (Bower, 2008).

One of the mechanisms that is commonly studied as a possible link between depression and breast cancer disease outcome is the up-regulation of pro-inflammatory cytokines (Schieper et al., 2005). Studies have shown that pro-inflammatory cytokines,

when released by macrophages, interact with neurotransmitter metabolism, neuroendocrine functioning, synaptic plasticity, and behavior, all of which are characteristics of Major Depressive Disorder (MDD) and depressive symptoms (Raison, 2006). In healthy women, severity of depression has been associated with pro-inflammatory cytokine up-regulation, specifically interleukin-1-beta (IL-1 β), IL-6 and tumor necrosis factor- α (TNF- α) (Raison, 2006, Suarez et al., 2008).

Many mechanisms have been proposed to link depression and pro-inflammatory cytokine up-regulation, however many are not well understood. Miller et al. (2003) proposed that as a consequence of depression, cancer patients may gain weight due to lack of activity. They found that excess weight leads to increases in adipose tissue which releases large amounts of IL-6 (Miller et al., 2003). Adipose tissue can also release IL-1 and TNF- α although they were not measured in that study (Coppack, 2001). Another study looked at exhaustion as a symptom of depression and found that it too correlated with greater levels of pro-inflammatory cytokines among angioplasty patients (Appels et al., 2000). Smoking, also a common behavior associated with depression, has been suggested as a factor due to its significant association with increased levels of IL-6 and TNF- α (Kop, 2002; Tapi et al., 1995).

On a genetic level, Myint & Kim (2003) suggest a neurodegenerative hypothesis of depression involving the interaction between cytokines and serotonin through *indoleamine-2,3-dioxygenase* (IDO). It has been reported that cytokines may decrease expression of *5-HT*, a serotonin receptor gene, through increasing the activity of IDO. IDO metabolizes tryptophan, which is the precursor for *5-HT*, to neurodegenerative quinolinate and neuroprotective kynurenate (Myint & Kim, 2003). When there is more

neurodegenerative quinolinate in the brain, brain regions that affect stress-related neuroendocrine functioning, like the hippocampus, begin to degrade (Myint & Kim, 2003). This may result in major depression or treatment-resistant depression (Myint & Kim, 2003). A 2000 study by Bremner and colleagues used Magnetic Resonance Imaging (MRI) to show that patients with a history of depression had a 19% smaller left hippocampal volume than matched comparison subjects. This would support the neurodegenerative hypothesis, however the authors of this study propose different explanations, suggesting that decreased hippocampal volume might be from increased glucocorticoids during depressive episodes or reductions in neurotrophins. Neurotrophins are responsible for survival and repair of neurons (Kaplan & Miller, 2000). In addition, Bremner et al note it is possible that some people are born with lower hippocampal volumes making it a risk factor, which would go against the neurodegenerative hypothesis. Stronger evidence is needed to confirm the validity of the neurodegenerative hypothesis.

As a follow up to the Myint & Kim study in 2003, another set of researchers applied the neurodegenerative theory to the association between estrogen and depressive symptoms. It has previously been found that estrogen reduction in women as a result of menopause may lead to depressive symptoms (Freeman, 2010). It has additionally been found in women with post-partum depression that the administration of 17beta-estradiol, an estrogen supplement, significantly decreases depressive symptoms (Ahokas et al., 2001). Since estrogen can inhibit inflammation in the brain, Xu et al. (2003) proposed and confirmed that estrogen deficiency may lead to increased inflammation, activating IDO, and ultimately reducing 5-HT content (Xu et al., 2003). By reducing 5-HT the level

of serotonin in the body is also decreased. This may be why depressive symptoms emerge.

There are also theories which describe a neurocognitive pathway leading to the association between inflammation and social rejection related stress. In 2010, Slavich et al. recruited 124 healthy subjects and had each person complete a laboratory social stressor task. Saliva samples were taken in order to analyze inflammatory biomarkers IL-6 and a receptor for TNF- α , sTNF α RII over time during the activity (Slavich et al., 2010b). They found that there were significant increases in IL-6 and sTNF α RII from before, during, and after the task (Slavich et al., 2010b). A subsample of 31 subjects also completed an fMRI analysis. They found that greater increases in sTNF α RII, but not IL-6, were associated with greater activity in the dorsal anterior cingulate cortex and anterior insula (Slavich et al., 2010b). These two brain regions have previously been identified as being involved in negative affect and stress from social rejection (Eisenberger et al., 2007, Slavich et al., 2010b). Therefore, these findings suggest that social rejection related stressors may cause an up-regulation of the inflammatory response via a neurocognitive pathway. Furthermore, social rejection related stress may initiate negative cognitions and emotions which are hallmarks of depression (Slavich et al., 2010a)

Although many theories have been proposed which link depression and pro-inflammatory cytokine concentrations, another mediation pathway commonly described in the neuroendocrine literature is the glucocorticoid-resistance model. In this model, Miller et al. (2002) predict that chronic stress makes the immune system insensitive to the glucocorticoid hormones, such as cortisol, which function to inhibit the normal inflammatory response. Underlying this model is the theory that chronic stress causes

secretion of hormones which come from the hypothalamic-pituitary-adrenocortical (HPA) and the sympathetic-adrenal-medullary (SAM) axes (Miller et al., 2002). As the stressor and exposure to glucocorticoid hormones are maintained, white blood cells (e.g., monocytes) begin to respond by downregulating the expression of receptors that bind glucocorticoid (GC) hormones (Miller et al., 2002). With down regulation of these GC receptors, the immune system is less able to inhibit inflammatory responses (Miller et al., 2002). This model holds that there may be increased levels of pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α in serum of persons experiencing distress and/or depressive symptoms.

In 2004, researchers analyzed data from melanoma patients being treated with IFN- α , a cytokine which leads to upregulation of other pro-inflammatory cytokines and has been known to induce depressive symptoms (Capuron & Miller, 2004). They found patients exhibited two distinct behavioral syndromes within three months of beginning treatment. One was characterized by changes in mood, memory, and concentration and was called the mood/cognitive symptom cluster. The other was characterized by fatigue, psychomotor retardation, loss of appetite, and sleep disturbance and called the neuro-vegetative symptom cluster (Capuron & Miller, 2004). The experimenters found that within those exhibiting mood/cognitive symptoms, there was increased activity of the HPA axis, predicted to be from sensitization to the corticotrophin-releasing factor pathway in addition to changes in serotonin metabolism (Capuron & Miller, 2004). The neuro-vegetative symptoms were associated with decreased basal ganglia dopamine activity from changes in basal ganglia functioning (Capuron & Miller, 2004). In the end, the researchers suggested there may be two separate mechanisms explaining the

association between the pro-inflammatory cytokines and depression (Capuron & Miller, 2004).

In addition to the strong association between pro-inflammatory cytokines and depression, there is a strong relationship between pro-inflammatory cytokine up-regulation and cancer (Goldberg & Schweitzer, 2005). Overall, chronic inflammation tends to contribute to tumor development and progression as well as possibly increasing the risk of cancer recurrence (Coussens & Werb, 2002). In 2009, Pierce et al. conducted a study to see if circulating biomarkers of inflammation are prognostic of breast cancer survival in 734 breast cancer survivors. The biomarkers they specifically looked at were C-reactive protein (CRP) and serum amyloid A (SAA). CRP and SAA are both non-specific, hepatic proteins which are secreted in response to IL-1, IL-6, and TNF- α (Pierce et al., 2009). They are commonly used as markers for chronic, low-grade inflammation as well as cancer risk assessment (Pierce et al., 2009). In this study, the aim was to determine if 31 months after diagnosis, CRP and SAA can predict disease-free-survival and overall survival over a follow-up period of 4.1 years and 6.9 years, respectively. The results were that higher CRP and SAA concentrations were associated with reduced overall survival (Pierce et al., 2009). Higher concentration of CRP was also significantly associated with reduced disease-free survival while SAA was borderline associated (Pierce et al., 2009). This suggests that greater inflammation is a negative prognostic marker for breast cancer patients.

Given that inflammation predicts worse outcomes in breast cancer patients, it is important to understand how depressive symptoms can impact pro-inflammatory cytokines in this population (Goldberg & Schweitzer, 2005). Bouchard et al. (2016)

recruited early-stage breast cancer patients and examined the relationship between their serum pro-inflammatory cytokine levels and levels of depressive symptomology, measured by the Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1962). They found that when the magnitude of depressive symptoms is looked at on a continuum, as well as when women are categorized as meeting or not meeting the clinical threshold of depression, the association between depression and greater levels of IL-1- β and TNF- α was significant. The threshold for depression used in this study was a score of 7 on the HRSD as previously validated by Zimmerman et al. (2004) (Hamilton, 1962). There was also a trend showing greater concentration of IL-6 in those patients with higher levels of depressive symptoms compared to those with lower depressive levels. Many other studies have shown significantly greater IL-6 levels in depressed breast cancer patients. Soygur et al. (2007), found that IL-6 levels were significantly higher in patients with both MDD and breast cancer than in patients with breast cancer alone, MDD alone, or healthy controls. The conclusion from these studies is that patients with breast cancer and depression have an increased concentration of pro-inflammatory cytokines IL-6, IL-1- β and TNF- α compared with individuals who are not depressed or otherwise healthy.

RAGE, S100A8/A9 Ligands, and Inflammation

Neuroendocrine processes related to the sympathetic nervous system (SNS) and HPA axis have been proposed as mediators of the association between psychological adversity and inflammation in many populations, including cancer patients (Antoni et al., 2006; Cole et al., 2015). Another potential endocrine process that may explain the association between depression and pro-inflammatory cytokines in breast cancer are those related to the receptor for advanced glycation end products (RAGE) (Yin et al.,

2013). It has been implicated in a number of acute and chronic inflammatory diseases and is beginning to emerge as an important factor in explaining the relationship between inflammation and cancer (Riehl, Angel, & Hess, 2009; Hudson & Lippman, 2018).

RAGE is a transmembrane protein and multi-ligand cell surface receptor part of the immunoglobulin super-family of proteins (Neeper et al., 1992; Yin et al., 2013). RAGE's ability to recognize unrelated exogenous and endogenous ligands based on their charge and multimeric state make it a pattern recognition receptor (PRR) (Chavakis et al., 2003). PRR's are an important aspect of the innate immune system as they are able to detect pathogens and problematic endogenous signals and signal the inflammatory response (Suresh & Mosser, 2013).

RAGE binds a number of endogenous and exogenous ligands. Some of the endogenous ligands include HMGB1 and S100/Calgranulins (Hudson & Lippman, 2018). HMGB1, also called high mobility group 1 or amphoterin, is secreted by monocytes and neutrophils (among other immune cells) in response to TNF- α , IL-6, CpG DNA, and endotoxin lipopolysaccharide (Hori et al., 1995). HMGB1 activates the pro-inflammatory response by binding to RAGE on cell types such as neutrophils, monocytes, endothelial cells, and cancer cells (Hori et al., 1995; Kang et al., 2013). The S100 family consists of 21 small proteins with similar structures but diverse functions and expression patterns (Donato, 2001). These proteins act as cell sensors, cell growth and differentiation modulators, and actin cytoskeleton organizers (Donato, 2001). In inflammatory states, S100 proteins are released in a similar manner to cytokines from a variety of cell types (Hoffman et al., 1999). Though there are many proteins in the S100 family, only proteins which form homo or hetero-dimers need to bind to RAGE, such as S100A8 and S100A9

(Leclerc, Fritz, Vetter, & Heizmann, 2009; Ostendorp et al., 2007). S100A8 and S100A9 are members of calgranulin subset of S100 proteins and are found mainly in the extracellular space as a heterodimer called calprotectin (Perera, McNeil, & Geczy, 2010). Calgranulin proteins are expressed and released in myeloid cell types, and calprotectin is expressed by neutrophils (Rammes, 1997).

In a recent review by Hudson & Lippman (2018), the authors discussed the effect of RAGE on number of inflammatory diseases including diabetes, cardiovascular disease, neurodegenerative conditions, and cancer. When diabetes in uncontrolled advanced glycation end-products accumulate, leading to a number of vascular complications (Hudson & Lippman, 2018). In 1996, Watier et al. found that by blocking RAGE in rats with type I diabetes, they were able to block increases in vascular permeability. Thus, blocking RAGE may be a target for preventing vascular complications from diabetes (Lippman & Hudson, 2018). In addition, a major cause of mortality in those with diabetes is cardiovascular disease due to atherosclerosis (Hudson & Lippman, 2018). A number of studies have shown that RAGE blockade in diabetic and nondiabetic apoE-null mice, protects against atherosclerosis development (Wendt et al., 2006; Soro-Paavonen et al., 2008, Harja et al., 2008). RAGE inhibition has also been shown to speed up wound healing, and reduce the development of capillary lesions in the retina (Barile et al., 2005; Goova et al., 2001) Thus, a treatment involving RAGE inhibitors may potentially reduce complications from diabetes (Hudson & Lippman, 2018).

In 2013, Yin et al. began to investigate whether S100A8/A9 binding directly to RAGE relates to breast cancer tumor growth and metastasis. This research group found that RAGE, by direct binding with S100A8/A9, led to the migration and chemotaxis of

breast cancer cells (Yin et al., 2013). These researchers also found that RAGE induced epithelial-mesenchymal cell transition (EMT) through the NF- κ B signaling pathway (Yin et al., 2013). In addition, this group applied their findings to human tissue of invasive ductal carcinoma. They found that RAGE was expressed in 15 out of 156 healthy mammary glands, and 205 of 268 invasive ductal carcinoma tissues ($P=0.000$) (Yin et al., 2013). In addition, there were significant associations between RAGE expression up-regulation and tumor differentiation ($P=0.027$), lymph node metastasis ($P=0.012$), and distant metastasis ($P=0.020$) (Yin et al., 2013). Yin and colleagues concluded that RAGE is over-expressed in cancerous tissue and may lead to greater risk of metastasis (Yin et al., 2013).

In 2008, Ghavami and colleagues aimed to determine the mechanism by which S100A8/A9 leads to cell growth via RAGE activation and signaling. Their first finding was that the cell growth promoting properties were only present when the concentration of S100A8/A9 was below 25 μ g/ml (Ghavami et al., 2008). Above that, the heterodimers have cell death related properties making them similar to cytokines with two opposite functional abilities (Ghavami et al., 2008). This may be because sRAGE, which competes with RAGE to bind S100A8/A9, is released with increased heterodimer concentrations (Ghavami et al., 2008). To confirm that S100A8/A9 was binding specifically to RAGE, they performed a number of experiments where RAGE was blocked before cells, including the MCF-7 and MDA-MB231 breast cancer cell lines, were treated with S100A8/A9. They found that without RAGE the cell-promoting properties of S100A8/A9 were ameliorated (Ghavami et al., 2008). Their next set of experiments demonstrated that p38 and p44/42 mitogen-activated protein kinases (MAPK) were phosphorylated when

cells were treated with S100A8/A9. They also found that the treatment caused activation of the NF- κ B signaling pathway (Ghavami et al., 2008). These findings may provide key evidence as to how RAGE-dependent signaling pathways may act to increase pro-inflammatory cytokines in breast cancer patients.

MAPK are a series of serine/threonine protein kinases which have been found to mediate a number of biological processes including the biosynthesis of pro-inflammatory cytokines (Kaminska, 2005). The p38 MAP kinase cascade previously identified is independent of two other major cascades found in humans, ERK1/2 and JNK (Kaminska, 2005). When a MAP kinase is activated, transcription factors present in either the cell's nucleus or cytoplasm are phosphorylated leading to expression of a target gene (Kaminska, 2005). The p38 MAP kinase is associated with gene expression of a number of cytokines, including IL-1, IL -6, and TNF- α (Kaminska, 2005). The other major signaling pathway activated by S100A8/A9 RAGE binding is the NF- κ B signaling pathway (Ghavami et al., 2008). When a cell is not stimulated, NF- κ B protein complexes are bound to inhibitory I κ B proteins and trapped in the cytoplasm of the cell (Salminen et al., 2007). Upon stimulation, I κ B proteins are phosphorylated, and then ubiquitinated. This breaks them down into proteasomes (Salminen et al., 2007). Following these steps, the NF- κ B complex is translocated to the cell nucleus and aids in transcription of genes, including those of pro-inflammatory cytokines and RAGE (Salminen et al., 2007, Tobon-Velasco, Cuevas, & Torres-Ramos, 2014).

In summary, it has been found that S100A8/A9 activation of RAGE can induce NF- κ B signaling pathways and MAPK signaling pathways, which promote inflammation via up-regulation of the proteins IL-1, TNF- α , and IL-6 among others (Hermani, 2006,

Kaminska, 2005). To date, no studies have looked at whether circulating levels of S100A8/A9 ligands might act as an intermediary between depressive symptoms and pro-inflammatory cytokine up-regulation in any human studies.

Understanding RAGE activation by S100A8/A9 and its involvement in inflammation is important to understanding the link between depressive symptoms and inflammatory cytokines because depression may be involved in increasing the concentration S100A8/A9. In December 2017, Jonassen et al., found that by exposing coronary artery disease patients to acute stress, there was a rapid increase in S100A8/A9 concentration which remained elevated 24 hours later (Jonassen et al., 2017). This study also measured diurnal cortisol levels, which were associated with the increase in S100A8/A9 concentration (Jonassen et al., 2017). The authors of this article proposed that since flattening of diurnal cortisol levels are associated with HPA axis dysfunction, and HPA dysfunction is associated with glucocorticoid receptor resistance, the negative relationship between glucocorticoids and S100A8/A9 leads to increased ligand concentration (Jonassen et al., 2017). Therefore, depression may be involved in the increase in S100A8/A9, which via activation of RAGE might increase pro-inflammatory cytokines.

Proposed Study

Evidence for a link between depression and inflammation is rapidly growing. Less is known about the ways that depressive states may increase the likelihood that cellular sources of RAGE ligands (e.g., s100A8/A9) become activated and promote cytokine

production via RAGE on target cells. It is possible that depressive states activate the sympathetic nervous system, which leads to β -adrenergic signaling and possible increases in tumor associated macrophages via the development of precursor monocytes in the bone marrow. (Cole et al., 2015). These cells are often then recruited to the tumor microenvironment. S100A8/A9 ligands are produced by immature myeloid cells found in the tumor microenvironment including monocytes, granulocytes, and myeloid-derived suppressor cells (MDSC's) (Ichikawa et al., 2012). In 2006 Suryono et al. examined the effect of norepinephrine and cortisol in a human monocyte cell line. They found that epinephrine lead to increased expression and production of S100A8/A9 mRNAs via β -adrenergic signaling (Suryono et al., 2006). This study provided evidence that stress may regulate S100A8/A9 levels in monocyte cells. It is therefore plausible that S100A8/A9 expression, induced by stress, may bind and activate RAGE and the NF- κ B signaling pathway, increasing the concentration of IL-1, IL-6, and TNF- α . If increased glucocorticoid concentrations lead to glucocorticoid receptor resistance in myeloid cells and increased NF- κ B expression, this provides further evidence that once the inflammatory process begins, regulation is suboptimal by dysfunctional glucocorticoid receptors and pro-inflammatory cytokines begin to be overexpressed. Thus, the reason that depressive symptomology may lead to inflammatory responses via S100A8/A9 could be due to increased effusion of S100A8/A9 expressing myeloid cells from the bone marrow to the tumor microenvironment, as well as dysregulation of cytokine production due to resistance of glucocorticoid receptors in the myeloid cells. Although no literature currently exists on the topic, I proposed that depressive symptomology may be associated with greater S100A8/A9 concentrations in breast cancer patients and may help to explain

the previously documented association between depressive symptoms and serum pro-inflammatory cytokines in this population (Bouchard et al., 2016). Given the strong evidence that depressive symptomology and inflammation, as well as S100A8/A9 ligands and inflammation are positively associated, I hypothesized that S100A8/A9 concentrations and therefore RAGE activation and signaling, as well as pro-inflammatory cytokines are both increased in breast cancer patients reporting greater depressive symptomology.

Aims and Hypotheses

Primary Aims:

Aim 1. To test whether depressive symptomology (measured categorically and continuously) in the 2 – 10-week post-surgical period relates to greater levels of pro-inflammatory cytokines after controlling for relevant covariates.

Hypoth 1.1. Women classified as depressed on the HRSD (score > 7) will show greater levels of serum IL-1- β , IL-6 and TNF- α vs women classified as not depressed (HRSD \leq 7) after controlling for BMI, time since surgery, type of surgery (mastectomy, lumpectomy) age, and stage.

Hypoth 1.2. Greater magnitude of depressive symptoms on the HRSD will relate to greater serum concentrations of IL-1- β , IL-6, and TNF- α after controlling for BMI, time since surgery, type of surgery (mastectomy, lumpectomy) age, and stage.

Aim 2. To test whether depressive symptoms (measured categorically and continuously) relates to greater levels of the RAGE ligands S100A8/A9 after controlling for relevant covariates.

Hypoth 2.1. Women classified as depressed on the HRSD will show greater levels of S100A8/A9 vs women categorized as not depressed after controlling for BMI, time since surgery, type of surgery (mastectomy, lumpectomy) age, and stage.

Hypoth 2.2. Greater magnitude of depressive symptoms on the HRSD will relate to greater serum concentrations of S100A8/A9 after controlling for BMI, time since surgery, type of surgery (mastectomy, lumpectomy) age, and stage.

Aim 3. To test whether S100A8/A9 ligand concentration serves as an intermediary in the relationship between levels of depressive symptomology (categorically and continuously measured) and pro-inflammatory cytokine concentrations.

Hypoth 3.1. The association between the magnitude of depressive symptoms and pro-inflammatory cytokine levels is attenuated when controlling for levels of S100A8/A9, after controlling for BMI, age, time since surgery, and stage. Depressive symptoms will demonstrate indirect effects on pro-inflammatory cytokines via S100A8/A9 levels.

Aim 4: To test the associations between HRSD scores, S100A8/A9 ligand concentrations, and pro-inflammatory cytokine levels at 12 months after the first measurements were taken. Specific hypotheses will be tested as above, with appropriate post-adjuvant covariates, and include the following.

Hypoth 4.1. Women classified as depressed on the HRSD (score > 7) will show greater levels of serum IL-1- β , IL-6, and TNF- α vs women classified as not depressed (HRSD \leq 7) after controlling for BMI, type of adjuvant therapy received

(chemotherapy, radiation, immunotherapy), time since completion of adjuvant therapy, and stage.

Hypoth 4.2. Greater magnitude of depressive symptoms on the HRSD will relate to greater serum concentrations of IL-1- β , IL-6, and TNF- α after controlling for BMI, type of adjuvant therapy received (chemotherapy, radiation, immunotherapy), time since completion of adjuvant therapy, and stage.

Hypoth 4.3. Women classified as depressed on the HRSD will show greater levels of S100A8/A9 vs women categorized as not depressed after controlling for BMI, type of adjuvant therapy received (chemotherapy, radiation, immunotherapy), time since completion of adjuvant therapy, and stage.

Hypoth 4.4. Greater magnitude of depressive symptoms on the HRSD will relate to greater serum concentrations of S100A8/A9 after controlling for BMI, type of adjuvant therapy received (chemotherapy, radiation, immunotherapy), time since completion of adjuvant therapy, and stage.

Hypoth 4.5. The association between the magnitude of depressive symptoms and pro-inflammatory cytokine levels at 12 month follow-up is attenuated when controlling for levels of S100A8/A9, after controlling for BMI, type of adjuvant therapy received (chemotherapy, radiation, immunotherapy), time since completion of adjuvant therapy, and stage. Depressive symptom magnitude will demonstrate indirect effects on pro-inflammatory cytokines via S100A8/A9 levels.

Aim 5. To test whether S100A8/A9 ligand concentration serves as an intermediary in the relationship between levels of depressive symptomology

(categorically and continuously measured) at the post-surgical time period and pro-inflammatory cytokine concentrations at the 12 month follow-up.

Hypoth 5.1 The differences in pro-inflammatory cytokine levels in depressed vs non-depressed women are attenuated when controlling for post- surgical levels of S100A8/A9, after controlling for BMI, age, time since surgery, and stage. Depressive symptomology will demonstrate indirect effects on pro-inflammatory cytokines via S100A8/A9 levels.

Hypoth 5.2. The association between the magnitude of depressive symptoms and pro-inflammatory cytokine levels is attenuated when controlling for post- surgical levels of S100A8/A9, after controlling for BMI, age, time since surgery, and stage. Depressive symptomology will demonstrate indirect effects on pro-inflammatory cytokines via S100A8/A9 levels.

EXPLORATORY AIM. To test if there are specific clusters of symptoms of depression (neuro-vegetative vs mood/cognitive) which relate to levels of pro-inflammatory cytokines and S100A8/9 ligand concentrations in the post-surgical and post-adjuvant periods. This aim was tested by substituting each of the symptom cluster scores with the total HRSD score. Since there are no clinical cut-offs for these cluster scores, HRSD was only tested as the magnitude of depressive cluster symptoms.

CHAPTER 2: METHODS

Participants and Procedures

Data for this thesis project came from a randomized controlled trial of a cognitive behavioral stress management intervention conducted in women with non-metastatic breast cancer. In the weeks after breast cancer surgery, women were randomized to either receive the 10-week Cognitive Behavioral Stress Management (CBSM) group therapy intervention, or engage in a one-day psychoeducation seminar within the corresponding 10-week period. Women in the South Florida area with Stage 0-III breast cancer were recruited 2 - 10 weeks post-surgery between December 1998 and February 2005. At the baseline measurement, women could not have had neo-adjuvant therapy prior to surgery or begun post-surgical adjuvant therapy. The assessments needed to occur after their surgery and before adjuvant therapy because these therapies often have effects on mood and biological processes including inflammation. They must also have been fluent in English, and had no co-morbid medical conditions, nor severe psychiatric illness. Specifically, women could not have been diagnosed previously with psychosis, panic disorder, or a major depressive episode. Once enrolled in the study the women completed self-report questionnaires as well as a structured interview assessing for depression, the HRSD (described below). Blood samples were taken from the patients in the late afternoon within one week of completing their psychological assessment. The blood samples were then processed and pro-inflammatory cytokine concentration and S100A8/A9 were determined and analyzed (Bouchard et al., 2016). Of the 240 women

participating in the parent trial only 59 women had stored serum samples available at baseline (post-surgery) and 12-month follow-up for cytokine and RAGE ligand concentrations.

Measures

Depressive Symptomology: Depressive symptomology was measured using the Hamilton Rating Scale for Depression (HRSD). This consisted of 17 items in an interview format (Hamilton, 1962). It was administered by a trained clinical psychologist and clinical psychology doctoral students who used a structured interview guide. For analyses of depressive symptoms as a continuum, the total HRSD score is used. For analyses that examined depressive symptomology as a binary variable, a previously validated cut-off score of 7 was used (Zimmerman et al., 2004). Those women with scores less than or equal to 7 were categorized as having low levels of depressive symptomology and those with scores greater than 7 were categorized as having elevated depressive symptomology. This questionnaire has been previously used in studies examining this cohort of breast cancer patients (Bouchard et al., 2016).

S100A8/A9 Concentration: Blood samples were taken by a licensed phlebotomist between 4pm and 6:30pm and delivered to the laboratory for processing the same day. Approximately 35ml of blood was obtained. Once received by the laboratory, the samples were spun down using centrifugation in order to separate the plasma, serum, and peripheral blood mononuclear cells. Samples were then cryopreserved for later analyses. An ultra-sensitive enzyme-linked immunosorbent assay (ELISA) was performed by

trained research personnel on the cryopreserved serum samples to quantify the concentration of S100A8/A9 ligands in the blood serum.

Cytokine Concentration: Blood sampling procedures described for S100A8/A9

concentration were also used to detect concentrations of pro-inflammatory cytokines.

Once serum was separated from the blood sample by centrifugation it was cryopreserved to be stored for later analyses. An ELISA was later performed on the cryopreserved sample to quantify the concentration of IL-1- β , IL-6, and TNF- α in the blood serum.

Covariates

The covariates chosen for the analysis at the post-surgical, pre-adjuvant time point included age, stage, BMI, type of surgery, and time since surgery. It has been found previously that as women age, their pro-inflammatory cytokine concentrations increase in a linear fashion (O'Conner et al., 2009). Therefore, age was controlled for in all analyses. Disease stage was controlled for because how far a woman has progressed in the disease before starting treatment is likely related to the concentration of pro-inflammatory cytokines. In this study, disease stage was categorized as non-invasive (Stage 0) or invasive (Stage I-III) (Bouchard et al., 2016). BMI was also an important covariate because greater weight is associated with greater adipose tissue, which is known to release pro-inflammatory cytokines (Miller et al., 2003). It was also important to control for time since surgery and the type of surgery received. This is because it is possible the surgery itself had an effect on pro-inflammatory cytokine levels given the nature of having and healing from a surgery (Wang et al., 2015). The covariates chosen for the analysis at the post- adjuvant time point included BMI, type of adjuvant therapy received (chemotherapy, radiation, immunotherapy), time since completion of adjuvant therapy,

and stage. Time since completion of adjuvant therapy as well as type of adjuvant therapy were important to control for at this time point because they may have significant effects on the levels of pro-inflammatory cytokine between the women.

Statistical Analyses

All statistical analyses were completed using SPSS 24 (SPSS, Chicago, IL). An analysis comparing study variables in the total population of women in the parent study (n=240) vs. the women included in the present study (n= 59) were conducted to ensure that there are no significant differences both in terms of demographics and study variables. Only 59 of this group of 240 women had stored serum samples to obtain biomarkers from at both the post-surgical and 12-month time points, but otherwise did not differ systematically from the total sample.

All study variables were analyzed to ensure normality and to correct for outliers. Coefficient alphas were computed for the HRSD total scores and the two depressive symptom cluster scores to document that there was adequate internal consistency in the total depressive symptom scores and the neuro-vegetative symptom and mood/cognitive symptom cluster scores.

To compare those with high versus low depressive symptomology on cytokine concentrations and S100A8/A9 ligand levels, an analysis of covariance was used. Multiple regression analyses were used to analyze the associations between the magnitude of depression symptoms and cytokine concentrations, and the magnitude of depression symptoms and S100A8/A9 ligand concentrations. To determine if S100A8/A9 ligand concentrations acted as an intermediary between depressive symptomology and

levels of pro-inflammatory cytokine concentrations, the PROCESS subprogram for SPSS (Hayes A. F., 2012) was used to examine direct, total, and indirect effects. Bootstrap confidence intervals were used to interpret the indirect effect. In general, bootstrapping in a mediation analysis is used to create a sampling distribution, which is used to create confidence intervals (Hayes, 2013). In this study a bias corrected interval was used. It was important to ensure that zero was not included in the confidence interval because if that was the case then it is possible that the indirect effect was equal to zero (Hayes, 2013). The bootstrapping approach to understanding the results of a mediation analysis was thought to work best in this study because of the small sample size. The small sample size may have resulted in greater non-normality and bootstrapping does not require an assumption of normality (Hayes, 2013). These analyses were repeated at the 12-month follow-up to address whether the association between depressive symptomology, S100A8/A9, and pro-inflammatory cytokines still exists following adjuvant therapy. In addition, analyses examining relationships between the three sets of variables over time were conducted using methods previously described. For the exploratory analyses, regressions were performed with both HRSD cognitive/affective symptoms and neuro-vegetative symptoms to analyze the association between each symptom cluster and S100A8/A9 ligand and cytokine levels. Finally, each depressive symptom cluster score was tested separately in the bootstrapping approach noted above.

CHAPTER 3: RESULTS

Preliminary Analyses

Sample Description

Of an initial sample of 240 women, 59 women had sufficient stored blood samples available for quantifying biomarkers as well as complete psychological data. Their ages ranged from 32 to 69, with an average age of 50.36 (SD = 8.38). These women were diagnosed with carcinoma in situ/Stage 0 (13.6%), stage I (42.4%), stage II (40.7%), or stage III (3.4%) breast cancer. Of these patients, 89.8% were receiving hormone therapy and 50.8% were pre-menopausal. The majority of the participants identified as White, non-Hispanic (72.9%), followed by Hispanic (20.3%) and Black (5.1%). The women were well educated as 11.9% completed high school, 27.1% completed 4 years of college, and 39% continued their education beyond 4 years of college. Additionally, 74.6% of the women held full-time jobs. There was a wide range of household incomes, falling between \$15,000 per year and \$300,000 per year.

In terms of the pathophysiological variables, women ranged from having 0 to 8 positive lymph nodes with the vast majority having 0 positive lymph nodes (81.4%). There were 32 women (54.2%) who had estrogen receptor positive cancers (ER+) and seven (11.9%) had estrogen receptor negative cancers (ER-). The remaining study patients did not know their ER status, and medical records were unavailable for documenting status. There were 21 women (35.6%) who had progesterone receptor positive (PR+) cancers and 10 (16.9%) who had progesterone receptor negative (PR-) cancers. The remainder of the women were unsure of their PR status, and medical records

were unavailable for documenting status. Of the 59 women in the study sample, 31 (52.5%) had received a lumpectomy and 28 (47.5%) received a mastectomy. All sample characteristics can be found in Table 1.

The low depressive and high depressive symptom groups differed significantly on BMI and race/ethnicity. The women in the high depressive category had significantly greater BMI ($M=28.24$) than the low depressive symptom group ($M=24.99$), $p = .021$. However, BMI was also not significantly correlated with continuous depressive symptom levels. BMI was not significantly correlated with any cytokine or S100A8/A9 concentrations at T1 nor T2. In addition, a chi square test revealed differences between high and low depressive symptom groups on ethnicity ($\chi^2 = 13.23$, $p = .001$). There were more non-Hispanic white women in the low depressive symptom group ($n=33$, 89.2%) than in the high depressive symptom group ($n=10$, 45.5%). There were also more Hispanic women in the high depressive group ($n=8$, 36.4%) compared with the low depressive group ($n=4$, 10.8%).

Primary Analyses

Specific Aim 1

The first aim was to determine the association between depressive symptomology severity levels at 2-10 weeks post-surgery and levels of pro-inflammatory cytokines while controlling for age, stage, time since surgery, type of surgery, and BMI. To test the relationship between continuous depressive symptom severity scores and pro-inflammatory cytokines (IL-1 β , IL-6, and TNF- α), a linear regression was performed (Figures 1, 2, &3). Depressive symptom severity was significantly associated with IL-1 β

($\beta = .340, p = .011$) and TNF- α ($\beta = .288, p = .026$), however, it was only marginally significantly associated with IL-6 ($\beta = .242, p = .062$). To test the relationship between depressive symptom severity ratings, measured categorically, and pro-inflammatory cytokines, an analysis of co-variance (ANCOVA) was used, controlling for the same covariates noted above. There was a significant difference in levels of IL-1 β ($F(1, 52) = 5.27, p = .026$), IL-6 ($F(1, 52) = 5.70, p = .021$), and TNF- α ($F(1, 52) = 4.88, p = .032$) between those with and without depression at the post-surgical time point, showing greater levels of each cytokine in those classified as depressed vs those not depressed.

Specific Aim 2

The second aim of this study was to determine the relationship between depressive symptom severity levels at 2-10 weeks post-surgery and concentration of RAGE ligands S100A8/A9 while controlling for age, stage, time since surgery, type of surgery, and BMI (Figure 4). As a continuous variable, depressive symptom severity levels were significantly associated with S100A8/A9 levels, $\beta = .300, p = .038$. To determine if women categorized as depressed vs non-depressed differed on S100A8/A9 levels, an ANCOVA was performed using the same set of covariates. RAGE ligand levels showed only marginally significant differences between the two groups, $F(1, 52) = 3.33, p = .074$, with depressed patients showing greater S100A8/A9 levels. A summary of all regressions performed at T1 can be found in Table 2.

Specific Aim 3

The third aim of the study was to determine if S100A8/A9 levels acted as an intermediate variable between depressive symptom severity levels 2-10 weeks post-

surgery and pro-inflammatory cytokine levels while controlling for age, stage, time since surgery, type of surgery, and BMI. The indirect effect was tested using a bias corrected bootstrap estimation approach with 5,000 iterations. Bootstrapping is a procedure that uses resampling with replacement in order to better estimate the population parameter from the sample more effectively, especially when the sample size is small. The indirect effect between depressive symptom severity levels and IL-1 β via S100A/A9 level at 2-10 weeks post-surgery was .058, SE= .041, 95% CI [.0009, .1687]. The ratio of indirect to total effect was .1018. Since zero (0) does not lie within the confidence interval, it can be concluded there is a significant indirect effect. This serves as provisional evidence that S100A8/A9 may serve as an intermediary between depressive symptoms and IL-1 β levels. It is important to note directionality cannot be determined in this case. Therefore, a second analysis was completed to determine if the indirect effect was significant when IL-1 β was the independent variable, S100A8A9 as the intermediary variable and depressive symptom severity level the dependent variable. In this case, however, the indirect effect was .141, CI [-.0418, .4980] and therefore not significant. Thus the alternative pathway wherein s100A8/A9 level serves as an intermediary between circulating IL-1 β levels and depressive symptoms does not appear plausible.

The indirect effect between depressive symptom severity levels and IL-6 at the post-surgical time point was tested using a bias corrected bootstrap estimation approach with 5,000 samples. The indirect effect was .064, SE= .040, 95% CI [.0070, .1685]. The ratio of indirect to total effect was .9173. Since zero (0) does not lie within the confidence interval, it can be concluded there is a significant indirect effect, which provides provisional evidence that s100A8/A9 may serve as an intermediary variable between

depressive severity and IL-6. Since directionality cannot be determined, a second analysis was completed with IL-6 as the independent variable, s100A8/A9 as the intermediary variable, and depressive symptom severity as the dependent variable. The indirect effect was .202, SE= .171, 95% CI [-.0246, .6597], which was not significant, and suggests that the hypothesis that s100A8/A9 level serves as an intermediary between IL-6 levels and depressive symptoms is not plausible.

The indirect effect between depressive symptom severity levels and TNF- α at the post-surgical time point was .021, SE= .030, 95% CI [-.0142, .0957]. Since zero (0) lies within the confidence interval, it can be concluded there is no significant indirect effect. Since directionality cannot be determined, a second analysis was completed with TNF- α as the independent variable, s100A8/A9 as an intermediary variable, and depressive symptom severity as the dependent variable. The indirect effect in this direction was .127, SE= .142, 95% CI [-.0295, .5961] and therefore not significant.

Specific Aim 4

The fourth aim was to determine the association between depressive symptom severity, RAGE ligand concentrations, and levels of pro-inflammatory cytokines at a one year follow-up while controlling for BMI, age, type of adjuvant therapy received (chemotherapy, radiation, immunotherapy), and stage. To test the relationship between continuous depressive symptom severity scores and pro-inflammatory cytokines (IL-1, IL-6, and TNF- α), a linear regression was performed controlling for the covariates mentioned previously. Depressive symptom severity was not associated with IL-1 β (β = .058, p = .663), IL-6 (β = .038, p = .765), nor TNF- α (β =.104, p = .420) at the one-year follow up. To test the relationship between depressive symptomology severity ratings,

measured categorically, and pro-inflammatory cytokines at the one-year follow up, an ANCOVA was used. There was no difference in levels of IL-1 β ($F(1, 48) = .350, p = .557$), IL-6 ($F(1, 47) = .000, p = .993$), or TNF- α ($F(1, 47) = .274, p = .603$) between those with and without depressive symptomology at the one-year follow up.

To determine the relationship between depressive symptom severity levels at one year post-surgery and concentration of RAGE ligands S100A8/A9 while controlling for age, stage, BMI, and type of adjuvant therapy received (chemotherapy, radiation, and immunotherapy), another regression analyses was conducted. As a continuous variable, depressive symptom severity levels did not significantly relate to S100A8/A9 levels, $\beta = -.074, p = .630$. To determine if categorically there is a difference between those with and without depression, an ANCOVA was performed. There was no significant difference in RAGE ligand levels between the two groups, $F(1, 47) = .233, p = .632$.

Since there were no significant associations between depressive symptom severity scores and pro-inflammatory cytokines and RAGE ligands, it can be assumed that RAGE ligands cannot act as an intermediary variable between depressive symptom severity and pro-inflammatory cytokine concentration at the one year follow up time point. A summary of all regressions performed at T2 can be found in Table 3.

Specific Aim 5

The fifth aim of the study was to determine whether post-surgical S100A8/A9 ligand concentration mediated the relationship between depressive symptom severity levels post-surgery and pro-inflammatory cytokines at the one year follow up. The variables which were controlled for were age, stage, time since surgery, type of surgery,

BMI, post-surgical levels of pro-inflammatory cytokines, and type of adjuvant therapy received.

It was first important to determine whether post-surgical levels of depressive symptomology were associated with IL-1 β , IL-6, and TNF- α at the 12-month follow up. It was determined previously that depressive symptom severity was significantly associated with S100A8/A9 levels at Time 1. Depressive symptom severity at the post-surgical time point (time 1) was not associated with IL-1 β ($\beta = .032, p = .812$), IL-6 ($\beta = -.043, p = .733$), nor TNF- α ($\beta = -.034, p = .776$) at the one-year follow up (Time 2). However, since modern mediation analyses do not require the association between the independent and dependent variable, the indirect effect was tested nonetheless.

The indirect effect was tested using a bias corrected bootstrap estimation approach with 5,000 iterations. The indirect effect of post-surgical depressive symptom severity levels on IL-1 β concentration at 12 months via post-surgical S100A8/A9 levels was .023, SE = .026, 95% CI [-.0105, .0966]. This means there is not a significant indirect effect because 0 is included in the confidence interval. The indirect effect of post-surgical depressive symptom severity levels on IL-6 concentration at 12-months via post-surgical S100A8/A9 levels was .024, SE = .026, 95% CI [-.0063, .1063] and was also not significant. The indirect effect of post-surgical depressive symptom severity on TNF- α concentration at 12 months via post-surgical s100A8/A9 levels was .008, SE = .017, 95% CI [-.0175, .0556], and was also not significant.

Exploratory Aim

The final aim of this thesis was to test if there are specific clusters of symptoms of depression (neuro-vegetative vs mood/cognitive) which relate to levels of pro-inflammatory cytokines and S100A8/9 ligand concentrations in the post-surgical and post-adjuvant periods. After splitting the 17 depression items on the Hamilton Rating Scale for Depression into two groups, neuro-vegetative and mood/cognitive, a Cronbach's alpha test of internal consistency was performed for each symptom group using the results from the post-surgical time point. The neuro-vegetative symptom group included eight items ($\alpha = .66$) and the mood/cognitive group included nine items ($\alpha = .68$).

To determine the relationship between the two symptom clusters and pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α at 2-10 weeks post-surgery, a number of linear regressions were performed controlling for age, stage, time since surgery, type of surgery, and BMI. The neuro-vegetative symptom cluster was significantly associated with IL-1 β ($\beta = .274, p = .039$) and TNF- α ($\beta = .265, p = .038$), and marginally significantly associated with levels of IL-6 ($\beta = .236, p = .064$), with greater symptoms associated with greater cytokine level. The mood/cognitive symptom cluster was also significantly associated with IL-1 β ($\beta = .351, p = .010$) and TNF- α ($\beta = .266, p = .045$). This symptom cluster was not, however, significantly associated with levels of IL-6 ($\beta = .211, p = .112$).

To examine the relationship between the two symptom clusters and RAGE ligands at 2-10 weeks post-surgery, linear regressions were performed controlling for age, stage, time since surgery, type of surgery, and BMI. Both the neuro-vegetative

symptom cluster ($\beta = .270, p = .041$) and the cognitive/mood symptom cluster ($\beta = .283, p = .039$) were significantly associated with levels of S100A8/A9 at the post-surgical time point.

To evaluate the relationship between the two symptom clusters and pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α at the one-year follow up, a number of linear regressions were performed controlling for age, stage, type of adjuvant therapy, and BMI. The neuro-vegetative symptom cluster was not significantly associated with IL-1 β ($\beta = .059, p = .655$), IL-6 ($\beta = .054, p = .666$), nor TNF- α ($\beta = .065, p = .612$). The mood/cognitive symptom cluster was also not significantly associated with IL-1 β ($\beta = .047, p = .726$), IL-6 ($\beta = .014, p = .911$), nor TNF- α ($\beta = .128, p = .326$).

To determine the relationship between the two symptom clusters and concurrent levels of the RAGE ligands at the one-year follow up, linear regressions were performed controlling for age, stage, type of adjuvant therapy, and BMI. Neither the neuro-vegetative symptom cluster ($\beta = -.117, p = .440$), nor the cognitive/mood symptom cluster ($\beta = -.016, p = .919$) significantly predicted levels of S100A8/A9 at the post-adjuvant time point.

Additional linear regressions were performed to determine if each of the depression clusters at the post-surgical time point predicted S100A8/A9 and pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α at the one year follow-up. The first linear regressions examined the ability of each depression cluster to predict levels of S100A8/A9 at the one year follow up while controlling for age, stage, type of adjuvant therapy, BMI, and S100A8/A9 ligand concentrations at the post-surgical time point.

Neither the neuro-vegetative symptom cluster ($\beta = .037, p = .814$) nor the cognitive/mood

symptom cluster ($\beta = -.016, p = .920$) significantly predicted S100A8/A9 levels at the one year follow-up.

The final set of regressions examined whether each of the depression symptom clusters at the post-surgical time point predicted cytokines IL-1 β , IL-6, and TNF- α at the one-year follow up. Each regression controlled for age, stage, BMI, type of adjuvant therapy, and cytokine concentration at time 1. The cognitive/mood symptom cluster did not significantly predict the 12-month follow up levels of IL-1 β ($\beta = -.017, p = .899$), IL-6 ($\beta = -.078, p = .521$), nor TNF- α ($\beta = -.046, p = .697$). Similarly, the neuro-vegetative cluster did not significantly predict the 12-month follow up levels of IL-1 β ($\beta = .092, p = .448$), IL-6 ($\beta = .007, p = .954$), nor TNF- α ($\beta = -.005, p = .968$).

CHAPTER 4: DISCUSSION

It has been well established in the literature that there is a connection between depression and breast cancer prognosis. Though a multitude of theories exist, it has yet to be sufficiently examined what biological mechanisms are responsible for this connection. It is of utmost importance in the field to have an understanding of how depressive symptoms might influence a patient's prognosis as clinicians may seek to target depression early in the treatment process and mitigate a modifiable negative prognostic factor. One of the correlates of depression that is a possible driver of negative breast cancer prognosis is inflammation, and it is therefore imperative to understand how a person's depressive symptoms might relate to inflammation early in the treatment process.

The present study examines the relationship between depressive symptom severity, RAGE ligands S100A8/A9, and pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α , both at the 2-10 weeks post-surgery time point and one year later. The findings from this study build upon an existing literature on the associations between depressive symptoms and increased concentrations of pro-inflammatory cytokines in breast cancer patients and add to this by including measures of S100A8/A9 ligands. S100A8/A9 ligands interact with RAGE, a multi-ligand cell surface receptor which when activated leads to the migration and chemotaxis of breast cancer cells (Yin et al., 2013). Though no literature currently exists on the topic of depressive symptomology and S100A8/A9 ligand concentrations, I had predicted that there would be a positive correlation between the two. The current study provides the first documented evidence that the degree to which a breast cancer patient experiences depressive symptoms is positively related to the

amount of circulating S100A8/A9. This is an important finding as it illuminates associations between depression and a novel inflammation-related biomarker that may help to explain the previously reported association between depression early in the breast cancer treatment process and disease outcomes in these patients (Antoni et al., 2017, Giese-Davis, 2011, Hjerl et al., 2003, Satin, Linden, & Philips, 2009, Piquart & Duberstein, 2010).

Depressive Symptomology and Pro-Inflammatory Cytokines

Cross-Sectional Associations at Time 1

The first aim of the current study was to determine the relationship between depressive symptomology and levels of pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α at 2-10 weeks following breast cancer patients' surgical procedure. Consistent with the findings of Bouchard et al. (2016), the greater level of depressive symptoms a patient experienced, the higher their level of each of these pro-inflammatory cytokines. The dataset used in the present study was largely overlapping with the dataset used in the Bouchard et al. study, but the sample size in the current study was smaller given the need to include only patients with corresponding data for S100A8/A9 ligand concentrations. Specifically, I found that there was a significant association between depressive symptomology and IL-1 β and TNF- α and a marginally significant association with IL-6 when depressive symptomology was measured on a continuous scale. Moreover, when patients were categorized based on their score on the HRSD into depressed versus non-depressed groups (Zimmerman et al., 2004) all three cytokines were significantly higher in the depressed patients.

The glucocorticoid-resistance model posits that chronic stress and depression are associated with dysregulation in glucocorticoid control of inflammation via desensitization of monocyte glucocorticoid receptors and subsequent up-regulation of pro-inflammatory cytokine signaling and increases in circulating pro-inflammatory cytokines (Miller et al., 2002). The results found in this study are directly in line with this theory and suggest that glucocorticoid resistance may be more evident in depressed breast cancer patients. The current study is limited, however, in not providing empirical evidence for associations between HPA axis activity and either depressive symptoms or inflammation markers in this sample. Future work should include measures of HPA axis activity such as corticotropin-releasing hormone, adrenocorticotrophic hormone (ACTH) and cortisol, and leukocyte glucocorticoid receptor gene expression.

Furthermore, a previous study by Antoni et al. (2012), using a largely overlapping data set, has also provided evidence that greater levels of negative affect were associated with an up-regulation of leukocyte genes encoding for pro-inflammatory cytokines including IL-1, IL-6, and TNF- α . This study additionally examined the effect of CBSM treatment on both negative affect and leukocyte pro-inflammatory gene signaling and found that women who completed the treatment had significantly decreased negative affect and leukocyte pro-inflammatory gene expression (Antoni et al., 2012). Importantly, 50% of the genes down-regulated in the CBSM condition were those genes associated with greater negative affect at baseline, suggesting that a stress management intervention that reduces negative affect (including depressive symptoms) may also be hypothesized to down-regulate inflammatory signaling in circulating leukocytes. Antoni et al. (2012) used bioinformatics analyses, which inferred that this pattern of gene expression also

reflected an increase in glucocorticoid receptor signaling by increasing expression of genes with glucocorticoid receptor response elements. Bioinformatics analyses also led to the inference that CBSM also decreased NF-kB signaling, which may have played a role in the reduction of pro-inflammatory cytokine gene expression.

Antoni et al. (2012) examined gene expression of inflammatory cytokines with regards to negative affect, which differs from the current study which examines levels of depressive symptomology using the HRSD. Although negative affect and depressive symptomology are thought to be overlapping phenomena, it is possible that they are mutually exclusive and provide unique information about the mechanisms involved with glucocorticoid receptor signaling and pro-inflammatory cytokine gene expression. When Miller et al. (2002) proposed the glucocorticoid resistance model, the variable described was chronic stress, which though related to both depressive symptoms and negative affect, is a third variable which could also be overlapping or mutually exclusive. The relationship between chronic stress, negative affect, and depressive symptomology and glucocorticoid resistance needs further investigating to determine if there is a differential effect of each in relating to inflammatory processes, or if there is a variable in common (e.g. distress) which is ultimately responsible for the associations that have been found.

In 2017, another study was completed by Jutagir et al. which used a largely overlapping data set and examined how social well-being relates to pro-inflammatory and pro-metastatic leukocyte gene expression in women with breast cancer. They found that greater social well-being was associated with less leukocyte pro-inflammatory cytokine and pro-metastatic gene expression (Jutagir et al., 2017). In this study depressive symptomology was also associated with the expression of a similar set of genes in these

patients. However, the association between levels of depression (as measured by the HRSD) and leukocyte gene expression became non-significant when social well-being was controlled for, which indicates the possibility of shared variance between depression and social well-being in the ways that they relate to inflammatory processes (Jutagir et al., 2017).

It is important to consider when interpreting the results of the present study that depressive symptomology is a variable which is likely intertwined with a myriad of other psychological variables, such as stress, negative affect, and social well-being. It is difficult to separate out which variable might be responsible for inducing changes in leukocyte gene expression, pro-inflammatory cytokine levels, and S100A8/A9 ligand levels.

Cross-Sectional Associations at Time 2

Contrary to my hypotheses, the relationship between the pro-inflammatory cytokines and levels of depressive symptomology did not hold at the one-year follow up. There are a number of possibilities which may explain the difference in results at each time point. First, it is possible that there was a significant amount of variability with regards to timing and type of adjuvant treatment. The present study did not examine the specific type and intensity of chemotherapy or radiation that the patients were given, which may have impacted both their levels of depression and inflammation at 12 months. For example, a 2004 study by Puzstai et al. found that in breast cancer patients, delivery of chemotherapy drug paclitaxel increased pro-inflammatory cytokines IL-6, IL-8, and

IL-10 significantly. The present study also did not control for the number of days elapsed between the end of the adjuvant regimen and the 12-month follow-up point. It is possible that inflammation decreases as the post-adjuvant period increases, however further research needs to be done in this area. Future research is warranted to determine if the type, intensity, and timing of breast cancer treatment independently impacts the levels of pro-inflammatory cytokines over time. It is also important to note that a portion of the women in this study completed a course of Cognitive Behavioral Stress Management (CBSM) therapy between Time 1 and Time 2. This could have decreased the variability in measurements at T2, notably in those participants who were assigned to CBSM and completed the treatment. When CBSM was included in the analyses as a covariate, however, there was no difference in results, and depressive symptoms remained unrelated to inflammatory markers at 12 months.

Another factor that needs to be accounted for is level of fatigue. It has been found that at the end of treatment, 60% of women with breast cancer endorse significant difficulties with sleep and fatigue (Bower et al., 2011). In 2011, Bower et al. published a study which examined breast cancer patients' fatigue levels, depressive symptomology, and plasma inflammatory markers within three months of completing their primary breast cancer treatment. They determined that TNF- α signaling may cause fatigue in breast cancer survivors independent of depressive symptomology. This study, in contrast to the current study, found no association between TNF- α and depressive symptoms, as measured by the Beck Depression Inventory (BDI-II) (Bower et al., 2011). The authors suggest that although fatigue and depression commonly occur in breast cancer patients following primary treatment, they do not share a common underlying mechanism (Bower

et al., 2011). Future research should control for levels of fatigue. In addition to fatigue, another factor which may explain the inability to find associations between depression and pro-inflammatory cytokines at the one-year follow up is obesity. Obesity has been previously found to cause a chronic inflammatory response due to the association between increased adipose tissue and the immune system (Pe´rez de Heredia, Go´mez-Marti´nez, & Marcos, 2012). In 2005, Soon Park et al. found that in otherwise healthy individuals, weight and BMI was significantly correlated with IL-6, TNF- α , and CRP. This indicates that BMI may have maintained high levels of pro-inflammatory cytokines at the 1-year follow up, masking the effect of depressive symptomology.

Another important point to be made regarding the relationship between depression and pro-inflammatory cytokines is that the relationship may be bi-directional. There is a significant amount of literature which describes a phenomenon where pro-inflammatory cytokines, mainly IL-1 β and TNF- α , create what is called “sickness behavior”, which mimics many symptoms of depression, including loss of appetite, social withdrawal, and fatigue (Dantzer & Kelley, 2007). Many animal experiments have shown that IL-1 β and TNF- α administration induces a wide range of behavioral symptoms such as staying in the corner of their cages, reducing food and water intake, decreased motor activity, and increased pain sensitivity, many of which resemble depressive symptoms (Dantzer & Kelley, 2007).

There are a number of theories about biological mechanisms which may explain how pro-inflammatory cytokines may lead to sickness behavior and depressive symptoms in animals and humans. One common aspect of many theories is the involvement of indoleamine 2,3 dioxygenase (IDO). IDO is activated by pro-inflammatory cytokines and

blocks tryptophan through the kynurenine pathway. Tryptophan is a precursor of serotonin and therefore one common theory is that blocking tryptophan affects the neurotransmission and bioavailability of serotonin, thus inducing depression (Dantzer & Kelley, 2007). Another theory states that the pathway that degrades tryptophan generates quinolinic acid, an agonist of the glutamatergic N-methyl-D-aspartate receptor (NMDA) receptor (Müller & Schwarz, 2007). Dysfunction of the NMDA receptor is thought to be a precursor for depression, possibly due to glutamatergic overproduction (Müller & Schwarz, 2007). In addition to the tryptophan depletion hypotheses, it is also theorized that pro-inflammatory cytokines activate the HPA axis, which leads to over-activation of corticotropin releasing hormone circuits, commonly found in depressed patients (Holsboer, 2003).

Longitudinal Associations

The third aim of the study was to test whether post-surgical (time 1) depressive symptom levels could be used to predict post-treatment (12 month) levels of inflammatory cytokines. First I tested whether post-surgical T1 depressive symptoms predicted cytokine levels at 12 months (T2) while controlling for T1 cytokines levels. Separate regression analyses of each cytokine found no evidence for a longitudinal association between T1 depressive symptomology and T2 cytokines, in either uncontrolled analyses, or when covariates were added, suggesting that initial depressive symptom levels did not predict change in inflammatory markers over the one-year period. It is possible that the type of adjuvant therapy that the patients received and the specific timing of their therapy influenced the relationship between T1 depressive symptomology

and T2 inflammatory markers. A supplemental analysis to determine whether T1 depressive symptom levels predicted T2 depressive symptom levels was significant both with and without the addition of covariates. However, a paired-samples T-test was also completed to determine if there was a significant difference in levels of depressive symptoms at T1 and T2, and there was no significant difference between the two. One reason this might have been the case is that a portion of the participants in the study completed a Cognitive Behavioral Stress Management (CBSM) Intervention between T1 and T2. This could have affected the ability to predict change over time since whether or not participants received the intervention was not taken into consideration as part of the current study. When CBSM was added as a covariate along with age, stage, BMI, time since surgery, type of surgery, and T1 cytokine levels in a supplemental analysis, the significance of the association between T1 depressive symptoms and T2 cytokine levels remained insignificant. In 2016, Antoni et al. found that in a largely overlapping data set women who received the CBSM treatment had smaller 6-12 month change in a pattern of leukocyte gene expression referred to as conserved transcriptional response to adversity (CTRA) compared with the control group. The control group, consisting of women who did not receive CBSM, showed an increase in CTRA during the first year of treatment. A part of the CTRA response is expression of pro-inflammatory genes including IL-1, IL-6, and TNF- α , so it is likely that the effects of CBSM may have been to truncate the variance in cytokines at T2, causing it to be more difficult to observe depression x cytokine associations (Antoni et al., 2016).

Depressive Symptomology and S100A8/A9 Ligands

The next set of analyses examined the relationship between depressive symptomology and S100A8/A9 ligand concentrations. Previous research has found that S100A8/A9 ligands bind to RAGE and activate signaling pathways such as NF- κ B and MAP Kinase, both of which are inflammatory pathways (Ghavami et al., 2008, Kaminska, 2005). It was therefore hypothesized that depressive symptomology would also be related to S100A8/A9 ligand concentrations.

Cross-Sectional Associations at Time 1

The study found that in the post-surgical period, greater depressive symptomology, measured continuously, was associated with higher levels of S100A8/A9. When the relationship was examined using depressive symptom levels measured categorically, the result was marginally significant with depressed individuals having higher levels of S100A8/A9 than non-depressed. This result, similar to the depression x cytokine association, may be explained by the glucocorticoid resistance model. As noted previously, this model posits that immune cells respond to chronic stress responding by downregulating glucocorticoid receptors (GCs) on myeloid cells and other tissue, due to chronically elevated levels of circulating cortisol (Miller et al., 2002). GC's have an antagonistic relationship with nuclear factor κ B (NF- κ B), a transcription factor which promotes the expression of pro-inflammatory genes, as well as RAGE (Tobon- Velasco, Cuevas, & Torres-Ramos, 2014 & McKay & Cidlowski, 1998). When GC's and nuclear factor κ B interact, NF- κ B activation is inhibited (McKay & Cidlowski, 1998). Therefore, under chronic stress it is plausible that there is an upregulation of RAGE secondary to down-regulation of monocyte GC receptors. It was found in the present

study at the post-surgical time point that after controlling for age, stage, time since surgery, type of surgery, and BMI, that IL-1 β and IL-6 were significantly associated with levels of S100A8/A9 while TNF- α trended towards significance. Therefore, it is possible that depressive symptoms relate to cytokines and S100 through the similar mechanisms

Cross-Sectional Associations at Time 2

As was the case for the depression x cytokine analyses at 12 month follow-up there was no evidence that 12-month depressive symptomology was associated with S100A8/A9 levels in either uncontrolled analyses, or when covariates were added. In addition, when depressive symptomology was examined categorically the relationship between whether a person was depressed or not was also not related to levels of S100A8/A9. It is possible that the type of chemotherapy that the patients received, and the specific timing of their therapy impacted the relationship between post-treatment (T2) depressive symptom levels and S100A8/A9 levels. As a supplemental analysis, I also examined whether T2 cytokines and T2 S100A8/A9 levels were associated and found in uncontrolled analyses that there was no association between the two variables. When I added in covariates of age, stage, type of adjuvant treatment, and BMI I found that there similarly was no significant association between the S100A8/A9 and these cytokines.

Although the present study did not find significant associations between S100A8/A9 and pro-inflammatory cytokines at 12-month follow-up, there is extant literature on the connection between RAGE signaling and inflammation. S100A8/A9 is a ligand which binds to the RAGE receptor, leading to activation of signaling pathways (Yin et al., 2013). Both RAGE ligands and RAGE receptors have been implicated in breast cancer metastasis (Yin et al., 2013). In a 2018 review, Hudson & Lippman

describe RAGE signaling as a driving force in inflammatory diseases such as diabetes, cardiovascular disease, neurodegenerative diseases, and cancer. RAGE leads to atherosclerosis, for example, by mediating inflammatory cell recruitment to the atherosclerotic plaque (Chavakis et al., 2008, Hudson & Lippman, 2018). In terms of cancer, RAGE has been implicated in a number of metastatic processes including cell migration, tumor invasion, resistance to apoptosis, and proliferation (Hudson & Lippman, 2018). In 2016, Kwak et al., conducted a study to examine the role RAGE signaling plays in the metastatic process in breast cancer. They found that RAGE expression was increased in highly metastatic breast cancer cell lines of both humans and mice (Kwak et al., 2016). In addition, they found that RAGE expression increased levels of EMT transcription factors in a MEK-dependent manner (Kwak et al., 2016). MEK is involved in the MAPK signaling pathway, and MAPK is one of the signaling pathways which is activated by S100A8/A9 and RAGE interactions (Ichikawa et al., 2011 & McCain, 2013). MAPK activation allows it to bind to kinase targets, translocate into the nucleus, and go on to cause transcription of pro-inflammatory genes such as IL-1, IL-6 and TNF- α (Sun et al., 2013). Current literature on S100A8/A9 ligands shows that RAGE is highly expressed in invasive ductal carcinoma tissue and not as much in healthy tissue (Yin et al., 2013). Therefore, it may be possible that once the cancer cells have died (after adjuvant therapy), the healthy cells do not lead to increased signaling of S100A8/A9, and therefore there is also less RAGE expression. Future research should investigate how cancer treatments affect both RAGE expression, and S100A8/A9 ligand concentration and their associations with depression.

Intermediary Pathway Analysis

Hypothesized Pathway

Cross-sectional at Time 1

Since a significant cross-sectional relationship was found between depressive symptomology, S100A8/A9 ligands and pro-inflammatory cytokines, the next part of the analysis was to determine if S100A8/A9 played an intermediary role in the relationship between depressive symptomology and the increased levels of pro-inflammatory cytokines. It is important to note that this analysis was performed on cross-sectional data and therefore no causal conclusions can be drawn. However, it was hypothesized initially that depressive symptomology would relate to pro-inflammatory cytokine levels through S100A8/A9. This study found that at the 2-10 week post-surgical time point, a significant indirect effect existed for depressive symptomology on IL-1 β and IL-6 through S100A8/A9 concentrations. This provides provisional evidence that S100A8/A9 might serve as an intermediary variable in the association between increased depressive symptomology and pro-inflammatory cytokine signaling in post-surgical breast cancer patients.

Since RAGE is also a product of the pathways activated by S100A8/A9 and RAGE interactions, namely MAPK and NF-kB, it is likely that there is a cyclical mechanism at play (Salminen et al., 2007 & Tobon-Velasco, Cuevas, & Torres-Ramos, 2014). Therefore, I performed the analysis in the reciprocal direction by testing for an indirect effect between depressive symptoms at the post-surgical time point and pro-inflammatory cytokine concentrations through S100A8/A9 ligand concentration at Time

1. However, I did not find significant support for an indirect effect in this model. This might indicate that the direction of the pathway might begin with depression and end with inflammation.

Since the indirect effect between depressive symptomology and pro-inflammatory cytokines one year later via S100A8/A9 was not significant, a longitudinal intermediary pathway between depressive symptomology at the Time Point 1 (T1) and cytokines at the one-year follow-up (T2) was not tested. Therefore, the present study did not provide evidence for a temporal relationship among the study variables. One way to clarify the temporal relations among variables over time would be to repeat the study, but this time examining depression, cytokines and S100 levels at an additional time point between the post-surgical time point and one year, possibly at six months. By examining whether 6-month changes in depression predate (i.e., using 6 – 12 month changes) or follow from cytokine and S100 changes, and whether changes in S100 predate or follow from cytokines changes one could gather information on the temporal associations among variables. However, in order to make a causal conclusion an experiment would need to be conducted with depression being manipulated (behaviorally or pharmacologically) and cytokine and s100 levels being monitored at multiple time points thereafter.

Neuro-Vegetative and Cognitive/Emotional Symptom Clusters

As part of an exploratory analysis, depressive symptoms were split into two symptom clusters, a neuro-vegetative symptom cluster and a cognitive/emotional symptom cluster, and each cluster was related to inflammatory markers. Since depressive symptoms were only associated with inflammatory markers at the post-surgical time points (T1) in the present study these analyses were restricted to T1 cross-sectional tests.

Previous studies have shown that administration of cytokines to cancer patients induces both neuro-vegetative and mood/cognitive symptoms of depression (Capuron & Dantzer, 2003). Furthermore, Capuron & Dantzer (2003) found that whether patients received IL-2 or IFN- α as part of their cancer treatment, neuro-vegetative symptoms frequently preceded cognitive/emotional symptoms providing evidence of two different mechanisms (Capuron & Dantzer, 2003). Additionally, in 2013 a study that examined 2,861 individuals with anxiety and depression found that the relationship between depressive symptoms and IL-6, TNF- α , and CRP existed only for the somatic symptoms and not for the cognitive symptoms (Duijvis et al., 2013). Contrary to the results found by Duijvis et al., however, in the present study at the 2-10 week post-surgical time point both neuro-vegetative and cognitive/emotional depressive symptom clusters were significantly associated with IL-1 β and TNF- α . Partially in line with Duijvis et al. study, only the neuro-vegetative symptom cluster was (marginally) associated with IL-6 whereas the mood/cognitive cluster was not. Another study completed in 2008 by Lutgendorf et al. found similar results. In this study, the associations between each cluster of depression, IL-6, and diurnal cortisol rhythms were examined in epithelial ovarian cancer patients. They found that vegetative symptoms, but not overall depression or other non-vegetative depression symptoms, were associated with increased concentration of IL-6 and diurnal cortisol, measured as the area under the curve.

Lutgendorf et al. (2008) suggest that one possible reason that vegetative depressive symptoms are more strongly associated with IL-6 in cancer patients than emotional and cognitive symptoms is that IL-6 up-regulation from the tumor results in “sickness behavior” which mimics vegetative depressive symptoms. Sickness behavior

describes behavior that one might notice when a person is fighting an infection, such as weakness, anhedonia, anorexia, and difficulty concentrating (Dantzer, 2001). Sickness behavior is caused by pro-inflammatory cytokines such as IL-1, IL-6, and TNF- α though the individual effects of each cytokine on sickness behavior are not yet clear (Dantzer, 2001, Lutgendorf et al., 2008). In general, pro-inflammatory cytokines influence the central nervous system via permeability of the blood-brain barrier or stimulation of afferent fibers in the vagus nerve (Lutgendorf et al., 2008). It is also possible that increases in IL-6 activate the HPA axis and induce glucocorticoid resistance (Miller et al., 2002, Lutgendorf et al., 2008), which permits further or sustained inflammatory cytokine release by both circulating leukocytes, such as monocytes, and within brain tissue.

In the present study, both neurovegetative and emotional/cognitive depressive symptom clusters were significantly associated with S100A8/A9 ligand concentrations. In summary, these results do not provide any further evidence that the type of depressive symptom cluster differentially relates to the inflammatory markers measured in this study. This is in direct contrast to a number of studies completed in the past. It is possible that one reason that the results of the current study differ from past studies is that the majority of patients did not score in the clinical range of depression on the HRSD (62.7% of patients scored <7). Future research should compare patients who meet criteria for major depression and those with sub-clinical symptoms.

Limitations and Future Directions

The present study utilized previously collected cross-sectional data from two time points, one 2-10 weeks following major, curative surgery and one 12 months later. Due

to the cross-sectional nature of the study, neither causal relationships, nor temporal relationships could be found. Future research should aim to collect data at multiple time points in order to draw temporal conclusions about possible pathways which may exist. Animal-model research is warranted in order to isolate the effects of S100A8/A9 and pro-inflammatory cytokines as there are a large number of factors which cannot be controlled for in studies with humans. For example, mice with a knock-out for RAGE genes may be compared with wild-type mice to determine the role RAGE plays in relations between depressive symptomology/sickness behavior symptoms and pro-inflammatory cytokines over time. Additionally, it is unknown whether the women in this study had depressive symptomology prior to their diagnosis. Studies have yet to show whether the length of depressive symptomology affects the levels of pro-inflammatory cytokines. Another major limitation to this study was the large variability with respect to the type of treatment the patients received (i.e chemotherapy, radiation, hormone therapies), the cancer phenotype (ER/PR/Her2neu status) and the timing of measurements with regards to the time since surgery and the time since ending adjuvant therapy. The sample was also largely white and of middle-class, which limits the generalizability of the study. Another limitation of the current study is sample size. In order to detect a medium effect size of .15 with 5 predictors, the smallest model in this study, a sample size of 55 would be required to achieve a power of .80. The sample in this study of 59 gives a power of just over .80 which is adequate for 5 predictors but raises an issue for including additional controls. The sample size in this study was limited due to missing data on certain variables such as BMI and S100A8/A9 ligand levels. Future studies would benefit from including more participants.

Conclusion

This study demonstrated that greater depressive symptomology related to greater S100A8/A9 ligand concentrations, and pro-inflammatory cytokines IL-1 β , IL-6, and TNF- α among breast cancer patients 2-10 weeks post-surgery. These findings were not replicated at a 12 month follow-up. This is the first study to find evidence of a relationship between depressive symptoms and S100A8/A9 in post-surgical breast cancer patients. The results obtained in this study are consistent with the glucocorticoid resistance model, specifically in breast cancer patients following their surgical procedure, though inferences involving HPA axis mediation remain speculative. This study additionally adds to the existing literature on inflammatory processes and depression by including a measure of S100A8/A9 ligand concentration, a novel biomarker presently emerging as a key player in inflammatory processes and breast cancer prognosis. Future research is needed in order to better classify the directionality and nature of alternate pathways connecting depression to inflammation, and ultimately adverse breast cancer outcomes.

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Table 1. *Sample Characteristics*

Variable	Full Study Sample (n=59)		Elevated Depressive Symptoms (n=22)		Low Depressive Symptoms (n=37)		Elevated versus Low Depressive Symptoms, Group Differences		
	Mean	SD	Mean	SEM	Mean	SEM	F	df	p
Age	50.36	8.31	48.45	1.92	51.49	1.30	1.832	57	0.181
Education	15.81	2.58	15.36	0.695	16.08	0.343	1.066	57	0.306
BMI, kg/m ²	26.20	5.29	28.24	1.37	24.99	0.681	5.621	57	0.021
No. positive lymph nodes	0.64	1.69	0.91	0.446	0.490	0.231	2.464	57	0.357
HRSD Continuous T1	6.86	5.17	12.45	0.805	3.54	0.344	136.84	57	0.000
HRSD Continuous T2	6.21	5.24	8.82	1.25	4.61	0.687	10.239	57	0.002
Time from Surgery to Randomization	36.46	23.17	36.73	5.28	36.3	3.70	0.005	57	0.946
	n	%	n	%	n	%	χ^2	df	p
Race/Ethnicity							13.23	2	0.001
Black	3	5.1	3	13.6	0	0			
White	43	72.9	10	45.5	33	89.2			
Hispanic	12	20.3	8	36.4	4	10.8			
No Response	1	1.7	0	0	0	0			
Stage							6.001	3	0.112
0	8	13.6	4	18.2	4	10.8			
I	25	42.4	6	27.3	19	51.4			
II	24	40.7	10	45.5	14	37.8			
III	2	3.4	2	9.1	0	0			
Surgical Procedure							0.603	1	0.437
Lumpectomy	31	52.5	13	59.1	18	48.6			
Mastectomy	28	47.5	9	40.9	19	52.4			
ER Status							1.845	2	0.398
Positive	32	54.2	10	45.5	22	59.5			
Negative	7	11.9	4	18.2	3	8.1			
Unknown	19	32.2	8	36.4	11	29.7			
PR Status							2.246	2	0.325
Positive	21	35.6	6	27.3	15	40.5			
Negative	10	16.9	3	13.6	7	18.9			
Unknown	27	45.8	13	59.1	14	37.8			

Table 2. Descriptive Statistics for Immune Variables in Hi/Low Depressive Symptom subgroups at Baseline and 12 Month Follow-up

		Post- Surgical Time Point			One Year Follow-Up		
		Low Depressive Symptoms	High Depressive Symptoms	Overall	Low Depressive Symptoms	High Depressive Symptoms	Overall
Depressive Symptomology (Continuous)	N	37	22	59	40	18	58
	M	3.54	12.45	6.86	3.35	12.56	6.21
	SD	2.09	3.78	5.17	2.43	4.06	5.24
IL-1	N	37	22	59	40	18	59
	M	.643	2.21	1.23	1.55	1.32	1.57
	SD	2.80	3.13	3.00	2.47	2.37	2.51
IL-6	N	37	22	59	40	18	59
	M	3.52	5.18	4.14	3.95	3.96	4.05
	SD	2.93	2.44	2.86	2.67	2.60	2.69
TNF-α	N	37	22	59	40	18	59
	M	1.64	2.83	2.09	2.00	2.39	2.19
	SD	2.83	2.61	2.46	2.21	1.80	2.13
S100A8/A9	N	37	22	59	40	18	59
	M	8.25	8.65	8.40	8.08	8.20	8.14
	SD	.634	.571	.637	.588	.500	.568

All values for IL-1, IL-6, TNF- α are represented as ln(pg/ml).

Table 3. *Post-Surgical Regression Analysis of Depression and Inflammatory Measures*

Variable	S100A8/A9			IL-1			IL-6			TNF- α		
	B	SE B	β	B	SE B	β	B	SE B	β	B	SE B	β
Constant	7.035	.701	.300*	-5.230	3.273	.340*	-3.449	3.073	.242	-4.335	2.625	.288*
HRS D	.037	.016	.085	.197	.075	.329*	.134	.070	.242	.137	.060	.382*
Age	.006	.010	.230	.118	.048	.124	.100	.045	.295*	.112	.038	.202
Stage	.193	.112	.060	.493	.522	.012	1.170	.491	.310*	.655	.419	.035
Days Elapsed	-.002	.004	-.023	.002	.017	-.181	.015	.016	.122	.004	.014	-.200
Procedure Type	-.029	.168	-.040	-1.080	.786	-.070	-.729	.738	-.129	-.974	.630	-.058
BMI	.023	.016	.191	-.040	.075	-.181	-.006	.071	-.011	-.027	.060	-.058

*p<.05

*p<.05

Table 4. 12-month Regression Analysis of Depression and Inflammatory Measures

Variable	S100A8/A9			IL-1			IL-6			TNF- α		
	B	SE B	β	B	SE B	β	B	SE B	β	B	SE B	β
Constant	7.724	.918		-6.027	3.413		-4.859	3.599		-4.024	2.914	
HRSD	-.008	.016	-.074	.026	.060	.058	.019	.063	.038	.041	.051	.104
Age	.003	.011	.042	.087	.039	.302*	.077	.042	.241	.078	.034	.306*
Stage	.060	.116	.082	.977	.430	.313*	1.527	.454	.441*	1.260	.367	.457*
Tamoxifen	.063	.189	.050	.927	.701	.173	1.286	.739	.216	.262	.599	.055
Chemother- apy	.080	.287	.045	-.750	1.065	-.098	-1.789	1.123	-.211	-1.212	.910	-1.179
Radiation	-.179	.296	-.092	-1.888	1.099	-.228	-1.131	1.158	-.123	-1.110	.938	-1.151
BMI	.008	.016	.077	-.040	.075	.257*	.148	.061	.291*	.093	.050	.229

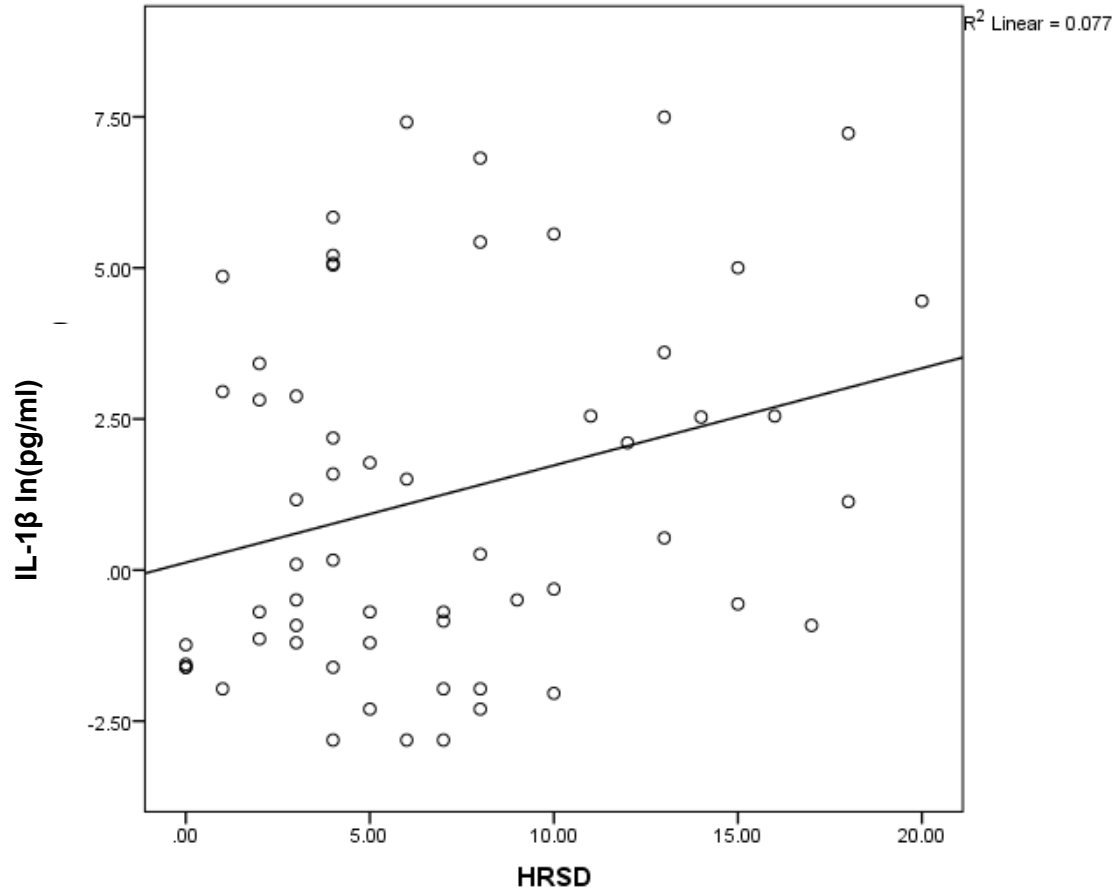


Figure 1. Association between HRSD score and IL-1 β at T1. Figure reflects unadjusted values. Cytokine level represented as ln(pg/ml). HRSD= Hamilton Rating Scale for Depression; IL= interleukin.

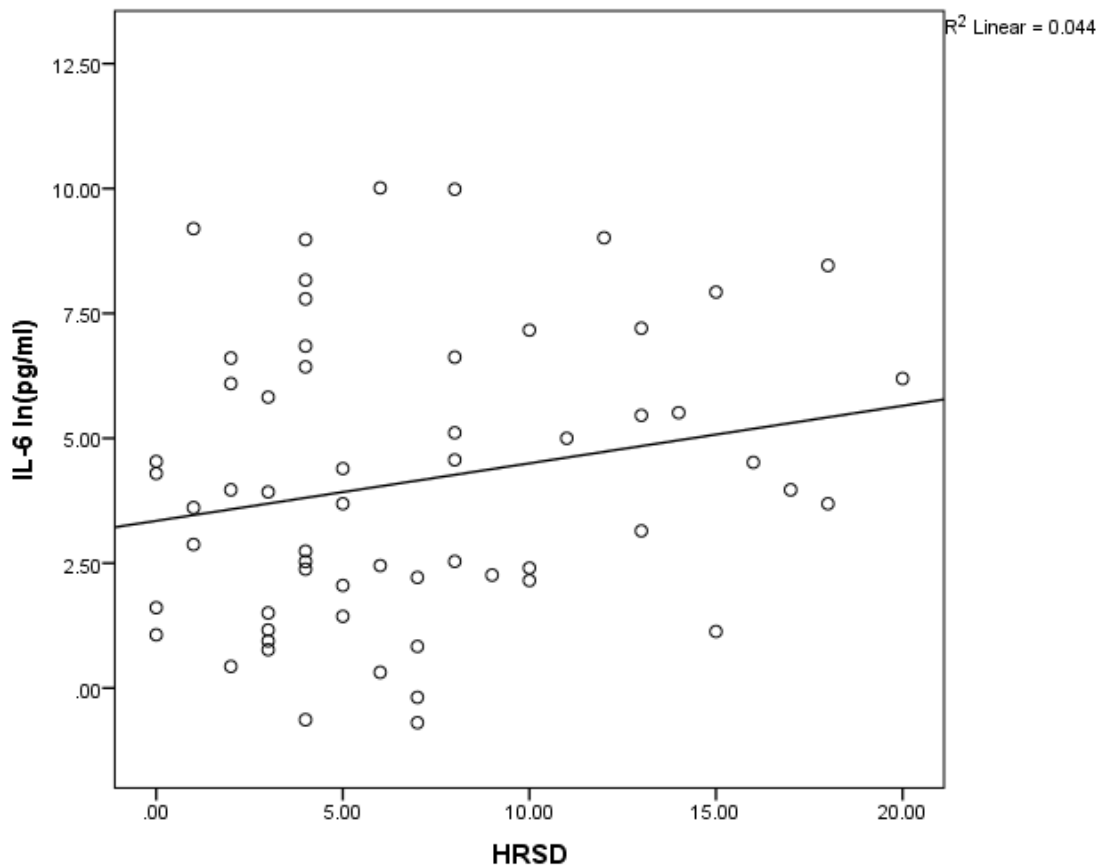


Figure 2. Association between HRSD score and IL-6 at T1. Figure reflects unadjusted values. Cytokine level represented as ln(pg/ml). HRSD= Hamilton Rating Scale for Depression; IL= interleukin.

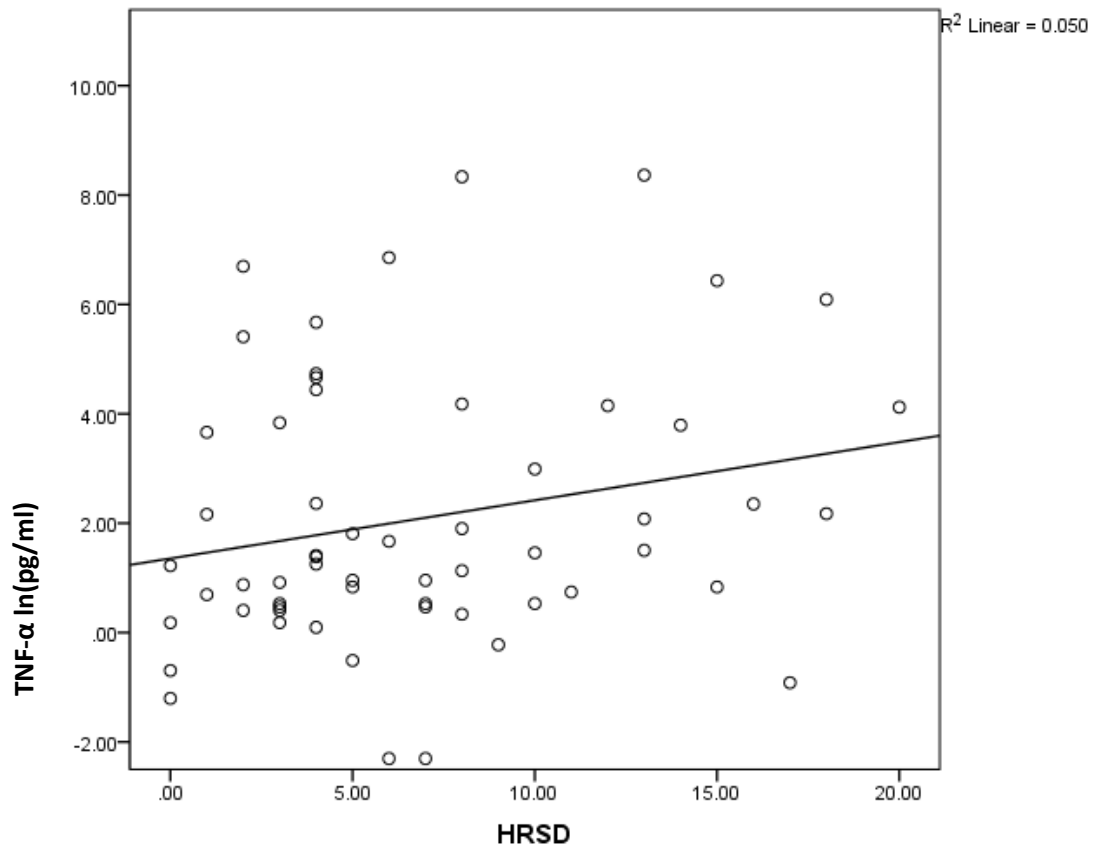


Figure 3. Association between HRSD score and TNF- α at T1. Figure reflects unadjusted values. Cytokine level represented as ln(pg/ml). HRSD= Hamilton Rating Scale for Depression; TNF- α = tumor necrosis factor- α .

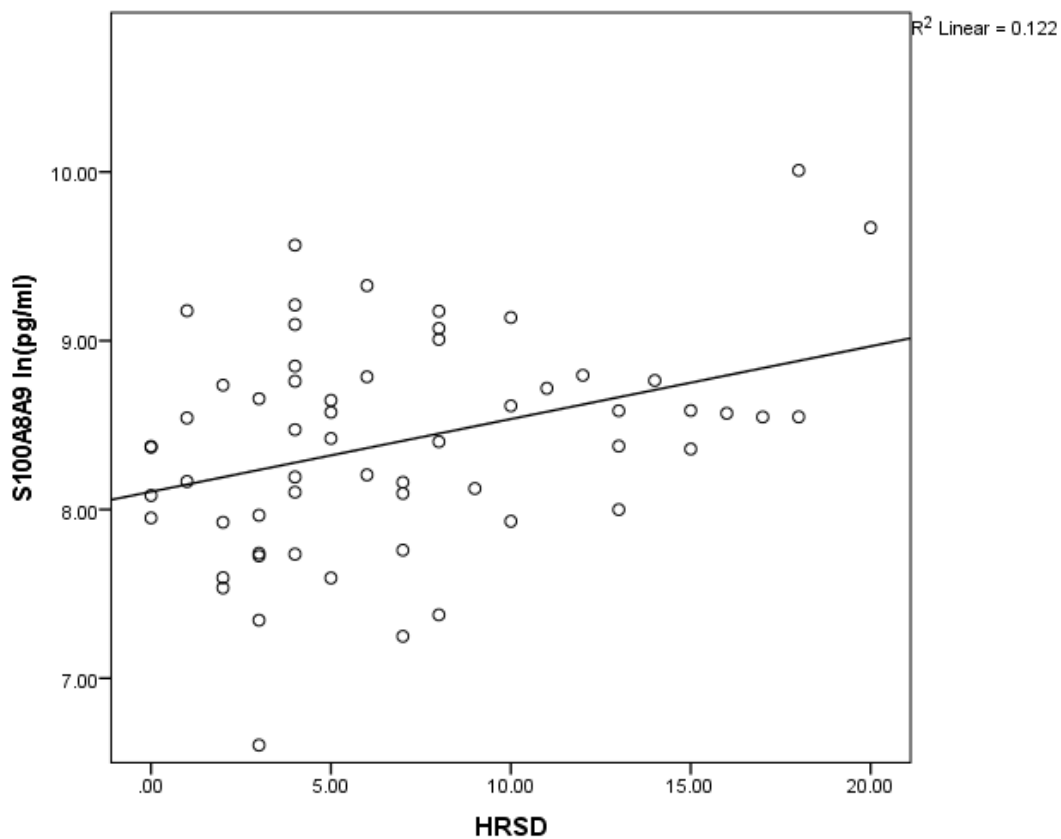


Figure 4. Association between HRSD score and S100A8/A9 at T1. Figure reflects unadjusted values. S100A8/A9 level represented as ln(pg/ml). HRSD= Hamilton Rating Scale for Depression.