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## **Psychoneuroimmunology and breast cancer: IL-6 - a possible but complex biomarker**

Degree Project in Medicine

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## Table of contents

Table of contents .....	1
Abstract.....	2
Introduction .....	4
Cytokines - part of our immune system .....	5
IL-6 .....	6
IL-6 in mood disorder .....	7
IL-6 and cancer .....	8
IL-6 and breast cancer.....	8
Aim .....	10
Methods .....	11
General methods .....	11
<i>Data collection and measures</i> .....	11
Subjects.....	11
<i>Inclusion criteria</i> .....	12
<i>Exclusion criteria</i> .....	12
Outcomes .....	12
<i>IL-6</i> .....	12
<i>Symptoms of mood disorder</i> .....	13
<i>Coping capacity</i> .....	13
<i>Personal growth</i> .....	13
Statistics.....	14
Ethics .....	15
Results .....	16
Study population, Baseline Data.....	17
IL-6 .....	17
Psychological outcomes.....	19
Correlations .....	19
Discussion.....	21
IL-6, physiological symptoms and disease stage .....	21
IL-6 and psychological symptoms .....	21
The way forward for inflammation-depression research .....	22
Limitations .....	23
Conclusion .....	25
Populärvetenskaplig sammanfattning .....	26
Acknowledgements.....	28
References .....	29

## Abstract

**Background:** Mood disorders are common in oncology patients and inflammation has been pointed out as a possible link between depression and cancer. The concentration of the cytokine IL-6 (Interleukin 6) has been showed to be increased in both depression and cancer. IL-6 is also correlated to overall survival in late stage cancer. This study investigates whether IL-6 correlates with symptoms of mood disorder, coping capacity, personal growth and common symptoms in women with breast cancer.

**Methods:** Data from 205 women diagnosed with breast cancer was included in the study. IL-6 concentrations were determined in serum and symptoms of mood disorder, coping capacity and personal growth was measured using Hospital Anxiety and Depression Scale, Sense of Coherence Scale and Posttraumatic Growth Inventory respectively. The Memorial Symptom Assessment Scale was used to measure both psychological and physiological symptoms. The data was analyzed in SPSS with Spearman's correlation coefficient and Mann-Whitney test. Patients with ongoing MDD (Major Depressive Disorder) were excluded.

**Results:** IL-6 positively correlated to physiological symptoms in late stage as well as cancer progress which is in line with earlier studies. Of the psychological factors measured, positive correlations were found between IL-6 and coping capacity, and IL-6 and "Appreciation for Life" in the women with early stage breast cancer.

**Conclusion:** Our results are in line with the idea that systemic IL-6 correlates with disease stage in breast cancer patients, but unlike earlier studies IL-6 does not correlate with symptoms of mood disorder. IL-6 might serve as a prognostic marker in more advanced stages of cancer but does not seem to have that function in earlier stages of breast cancer. A more refined understanding of how to best analyze the relations between our immune system and mood in cancer patients is desirable, as

recent research have suggested: omicsbased systems biomedicine methods seem promising for the field of inflammation-depression research.

Key words: psychoneuroimmunology, breast cancer, IL-6, mood disorder.

## Introduction

There is a growing scientific interest regarding the link between inflammation and mood disorders. New articles are being published on the subject every week and recent articles refer to anxiety and depression as psychoneuroinflammatory disorders (Miranda et al., 2017). However, the link is not fully understood. We know that there is a correlation between mood disorders and inflammation and it has been suggested that that it is a bi-directional relationship (Kohler et al., 2017; Passos et al., 2015; Rosenblat, Cha, Mansur, & McIntyre, 2014). Some of the unanswered questions are if there is a causality and if it is true for different types of mood disorders and in different types of patient groups. One group of patients that are likely to develop symptoms of mood disorders is the large group of people with a cancer disease. The cancer incidence, 2012, was 14.1 million worldwide, 25% percent of the cancers in women were breast cancer, which is the most common cancer in women (Torre et al., 2015). Receiving a cancer diagnosis and battling with the doubts concerning treatment, recurrence and mortality is an unimaginable stress. This mental and emotional stress combined with the stress of surgery, radiation and chemotherapy puts patients with cancer at a high risk of developing inflammation-induced behavioral changes (Miller, Ancoli-Israel, Bower, Capuron, & Irwin, 2008).

Depression is the type of mood disorder most commonly seen in patients with cancer, depending on type of instrument used to detect depression, the range in depression prevalence in cancer patients is 8-24% (Krebber et al., 2014). It is important to differentiate the patients with appropriate sadness from receiving a cancer diagnosis from the patients that are developing mood disorders and might need extra care and treatment.

Research resources are being used to find biomarkers for depression and pharmaceutical targets for treating mood disorders since it is one of the largest disease group in the world. One link between depression and cancer could be inflammatory driven, and this has been investigated in studies focusing on cytokines (Oliveira Miranda et al., 2014) ever since Maes in 1995 postulated the

hypothesis that IL-1 $\beta$  (Interleukin 1-beta) and IL-6 (Interleukin 6) may be responsible for some of the symptoms seen in depression (Maes, 1995). IL-6 has been shown to be elevated in cancer patients with depression compared to cancer patients without depression (Jehn et al., 2006).

In this study we investigate the relation between levels of systemic IL-6 and symptoms of mood disorder, symptoms, global symptom distress, symptom burden, coping capacity and posttraumatic personal growth in women treated for breast cancer.

### ***Cytokines - part of our immune system***

Our immune system is described to be divided in two sections: the innate and the adaptive. The innate immunity is made up of evolutionary conserved systems of recognition where innate leukocytes recognize conserved structures, exogenous Pathogen-Associated Molecular Patterns (PAMPs) and endogenous Danger-Associated Molecular Patterns (DAMPs), with their pattern recognition receptors (PRRs). PRRs binding to PAMPs or DAMPs activates an inflammatory response, pathogen associated or sterile respectively (Fleshner, Frank, & Maier, 2017). When local threats are recognized, cytokines are secreted and via the blood-stream activate cells in the liver to produce acute phase proteins, such as CRP for example. One of the strongest PAMPs is lipopolysaccharide (LPS), an evolutionary conserved molecule that is part of the outer membrane of gram-negative bacteria, and many studies on IL-6 response have been performed using LPS-injections as triggers. Common PRRs are the toll-like receptors (TLRs). TLR4 recognizes LPS and starts a signaling cascade with the transcription factor NF- $\kappa$ B, which then starts the transcription of cytokines such as IL-1 and IL-6, and tumor necrosis factor alfa (TNF- $\alpha$ ).

These cytokines further drive the inflammation both locally and systemically. Proinflammatory cytokines are responsible for the four cardinal symptoms of local inflammation: dolor, calor, rubor and tumor (pain, heat, redness and swelling), because they promote increased vascular permeability and cellular adhesion. However, IL-6 together with CRP can drive the systemic symptoms of

inflammation as well, such as fever, and increased heart and respiratory rate. These changes in bodily functions promote quickened healing (Slavich & Irwin, 2014). Cytokines can also affect our social behavior towards what is known as *sickness behavior*, described in animals 1988 by Hart. Sickness behavior includes a decrease in daytime activity, disturbances in sleep patterns, loss of appetite, and decreased interest in socializing, grooming, mating and hedonic behaviors (Hart, 1988). Slavich and Irwin (2014) points out the similarity between sickness behavior and the behavioral symptoms of depression. They suggest that “These effects thus argue for the possibility that cytokines may be able to induce major depression in humans”. They proposed a social signal transduction theory of depression, with the background mentioned above, where proinflammatory cytokines are the key mediators (Slavich & Irwin, 2014).

## **IL-6**

IL-6 is one of the human interleukins, which is a subgroup of cytokines. IL-6 is most commonly referred to as a pro-inflammatory cytokine, but it can also act as an anti-inflammatory signal molecule depending on its cellular binding sites. The IL-6 receptor (IL-6R) is expressed on hepatocytes, megakaryocytes, monocytes, macrophages, B cells and subtypes of T cells. IL-6/IL-6R forms a signaling complex via a glycoprotein 130 (gp130) homodimer, using a pathway called *classic signaling* and gives rise to an anti-inflammatory response. The IL-6R can also be in a soluble form, sIL-6R, and can then give rise to a pro-inflammatory response via binding to membrane bound gp130 which is ubiquitously expressed, using a pathway called *trans-signaling*. Lastly, when the complex IL-6/sIL-6R binds to the soluble form of gp130, trans-signaling will be blocked (Hodes, Menard, & Russo, 2016; Wolf, Rose-John, & Garbers, 2014). Because of these different responses, little information on the degree of activation of the downstream pathways of IL-6 activation is provided by the serum concentrations of IL-6 (Maggio, Guralnik, Longo, & Ferrucci, 2006). The trans-signaled pro-inflammatory activity of IL-6 decreases neutrophil and

favor mononuclear-cell accumulations, which is proposed to be one of the key factors of transitioning from acute to a more chronic inflammation (Lippitz & Harris, 2016).

IL-6 levels in healthy persons are usually of 1-5 pg/ml, but concentrations of g/ml have been measured in patients with sepsis (Wolf et al., 2014). IL-6 levels are higher in patients with cancer, in one review (Lippitz & Harris, 2016) the median of serum levels of IL-6 in cancer patients was 6.95 pg/mL versus 1.31 pg/mL in healthy control groups.

### ***IL-6 in mood disorder***

In a recent meta-analysis it was concluded that there is an association between IL-6 concentrations and major depressive disorder (MDD) (Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimaki, 2015) and another meta-analysis investigating inflammatory response to treatment in depression stated that IL-6 levels decrease with antidepressant treatment regardless of outcome in treatment result. It is also suggested that elevated levels of inflammation contribute to treatment resistance in depressed patients (Strawbridge et al., 2015).

Acute response of IL-6 to a traumatic event may be predicative of the severity of mood disorder. Pervanidou et al showed that an elevated plasma level of IL-6 in children after a motor vehicle incident was predicative for the development of posttraumatic stress disorder (PTSD) (Pervanidou et al., 2007). Hodes et al (2014) have reported that the acute response of IL-6 in rodents correlates with the severity of depression symptoms when put under chronic social stress. Furthermore, Khandaker et al described that children with higher IL-6 levels had a higher risk of developing depression and psychosis (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014). These are all examples of IL-6 being predicative of future development of mood disorders. Reichenberg et al also showed that LPS-induced higher levels of circulating inflammatory markers (TNF- $\alpha$ , TNF- $\alpha$  receptors, IL-6, and IL-1 receptor antagonists) are correlated with higher levels of anxiety and depression during the hours immediately following LPS-injection (Reichenberg et al., 2001). The



fact that approximately a third of the patients with MDD are non-responders to the treatments available today (Strawbridge et al., 2015) and that MDD is a leading cause of disability worldwide (Haapakoski et al., 2015) signals that there is a need for better therapeutic targets or a better understanding of the heterogeneity of this patient group. Current evidence suggests that part of this heterogeneity can be explained by inflammatory mechanisms (Saad et al., 2014).

### ***IL-6 and cancer***

Lippitz and Harris conclude in their review of cytokine patterns in cancer patients (2016) that IL-6 correlates with overall survival independent of the initial tumor histology. However, the IL-6 increase seems to be a late-stage cancer phenomenon. IL-6 is therefore suggested to be an independent possible predictor of survival in cancer.

Since IL-6 levels correlate with overall survival in cancer patients and it is suggested to reflect the cascade of interdependent cytokines involved in the paraneoplastic systemic inflammatory process (Lippitz & Harris, 2016), finding new ways to lower IL-6 or its effects could be a way to increase the overall survival in cancer patients. Therefore, it is of interest to see if coping capacity, psychological symptoms and personal growth correlate to IL-6.

### ***IL-6 and breast cancer***

Receiving a breast cancer diagnosis is both a physical and a psychological trauma. As is illustrated: systemic IL-6 levels have been described to correlate with overall survival in cancer patients, symptoms of depression and severity of post-traumatic stress. The effect of IL-6 on breast cancer cells is not fully understood. Studies have shown contradictory responses with both inhibitory effects on growth and proliferation, and growth promoting effects. To this Dethlefsen (2013) suggests in her review that high levels of IL-6, which is showed to correlate with overall survival in breast cancer patients, might just be a biomarker of obesity, physical inactivity and impaired metabolism (Dethlefsen, Hojfeldt, & Hojman, 2013) . Inflammation is lower in more physically

active people (Gleeson et al., 2011) and breast cancer survivors have a marked lower cardiorespiratory fitness than their inactive healthy controls (Jones et al., 2012).

# Aim

The aim of this study is to investigate whether IL-6 correlates with:

- symptoms of depression and anxiety (Hospital Anxiety and Depression Scale, HAD)
- Coping capacity (Sense of Coherence Scale, SOC)
- Symptoms, total symptom burden and global symptom distress (Memorial Symptom Assessment Scale, MSAS)
- Personal growth (Posttraumatic Growth Inventory, PTGI)

in women with breast cancer.

# Methods

## *General methods*

This study was done as a part of a larger intervention study, in which the purpose was to determine whether Mindfulness Based Stress Reduction (MBSR) therapy could influence mood disorders in women with breast cancer. It was carried out as a five-year longitudinal randomized controlled trial where the primary outcome, mood disorder, was measured using HAD (Hospital Anxiety and Depression scale) and some of the secondary outcomes were IL-6 levels in plasma and the results of questionnaires on the participants symptoms and health status (e.g. Sense of Coherence, SOC; Memorial Symptom Assessment Scale, MSAS; Posttraumatic Growth Inventory, PTGI).

This study is investigating the correlation between IL-6 and scores on HAD, MSAS, SOC and PTGI in women treated for breast cancer. We also explored the correlation between IL-6 concentration and recurrence in breast cancer.

### *Data collection and measures*

Socio-demographic data (age, marital status, children, living situation, employment status, occupation and educational level) and clinical characteristics (tumor characteristics, type of treatment and co-morbidity) was collected through chart reviews and interviews at baseline.

Blood sampling and self-reported responses with HAD, SOC, MSAS and PTGI used in this study was collected at base-line and after 3 months. The results from both observations was added to one group and further analyzed as one group.

## *Subjects*

Women diagnosed with early stage breast cancer or recurrence in breast cancer were consecutively enrolled from two surgical centers in Sweden, Sahlgrenska University Hospital and Skaraborgs Hospital. The women were asked to enroll and were informed by research nurses either at their first

follow-up appointment for receiving hormonal therapy or at the last treatment with chemotherapy. Written and oral information was given about the study. The participants could at any time choose to withdraw from the study. Written informed consent was gathered by the women, they were then randomized to one of the three groups: MBSR, active controls (MBSR, self-instructing program) or non-MBSR. More on the original intervention study on MBSR can be found in Kenne's study design (Kenne Sarenmalm et al., 2013).

### *Inclusion criteria*

Women diagnosed with breast cancer:

receiving hormonal therapy, or;

after completion of adjuvant chemotherapy, with or without radiotherapy

### *Exclusion criteria*

Presence of other advanced illness at diagnosis, and/or:

ongoing major depression

ongoing Herceptin therapy

previous use of the intervention (MBSR)

## **Outcomes**

### *IL-6*

Blood sampling was done in the morning by research nurses. The IL-6 concentrations in serum were determined by commercial high sensitivity ELISA kits (R&D Systems, Inc., Abingdon, UK) according to the instructions from the manufacturer.

### *Symptoms of mood disorder*

Symptoms of depression and anxiety was measured using the Swedish version of HAD (Hospital Anxiety and Depression scale). It is a 14-item questionnaire and contains two subscales: anxiety and depression, which can generate 21 scores each.

### *Coping capacity*

The professor Aaron Antonovsky developed the Sense of Coherence Scale (SOC), after his research for answers to how some people stayed healthy in the concentration camps of World War II. He suggested that the way people view their life affects their health, where a strong sense of coherence is associated with resources to cope with stressful life events (Eriksson & Lindstrom, 2005).

The Swedish version of SOC-13 items was used to evaluate the women's coping capacity. The questionnaire is made up of 13 questions with 7-point Likert-type scales to rate the answers.

Stronger sense of coherence is represented by higher scores (Antonovsky, 1993).

### *Symptom experience*

The Memorial Symptom Assessment Scale was used to capture the symptom distress and frequency. The scale includes 32 symptoms and scales on frequency, severity and distress of the symptoms in the women. *Total symptom burden* and *Global symptom distress index* is also generated by the scale (Portenoy et al., 1994).

### *Personal growth*

Posttraumatic Growth Inventory (PTGI) was used to measure positive life change and personal growth. It is a questionnaire with 21 items with 6-point Likert scales. The results can be divided into five aspects: Relating to others, new possibilities, personal strength, spiritual change and appreciation for life. The PTGI has been shown to be reliable in previous research of women with breast cancer. (Tedeschi & Calhoun, 1996)

## ***Statistics***

The statistical analyses were performed in IBM SPSS version 22. Spearman's correlation coefficient was used to investigate correlations, and between group comparisons was done using the Mann-Whitney test.

## Ethics

The study was granted ethical approval by the Ethical Committee, University of Gothenburg D:nr 499-9; 12/11/2009. Participation in the study was voluntary and the women could always choose to discontinue the study. All the data is deidentified and coded, and all the blood samples are kept encrypted, only the principal investigator of the original study have access to the code. For more ethical considerations regarding the original data please see the study design of the original study (Kenne Sarenmalm et al., 2013).



## Results

Two hundred and five women, of which 166 women with early stages of breast cancer and 39 was diagnosed with a recurrence in breast cancer, admitted our study at baseline and at the three months follow-up. Of the 39 women with recurring breast cancer they all started the intervention but five patients with recurrence left the study before the follow up at three months due to fatigue, two patients with recurrence died before the follow up and one patient did not do the follow up.

Resulting in a number of 31 women with recurrence assessed the follow up. The women with recurrence in breast cancer is in a second RCT-study on the effects of MBSR with the same design as the previous by Kenne, results are yet to be published. As different questionnaires, variables and blood samples deal with some drop outs and missing data the number of total observations including both baseline and follow-up varies between 340 and 410 for different sub-analysis. Due to missing data we do not know how many women were asked to participate in the study.

## Study population, Baseline Data

Table 1. Baseline demographic data and clinical characteristics of the study population.

Characteristic	N	%	Characteristic	N	%
<b>Marital status</b>			<b>Surgery</b>		
Married/cohabitation	154	75	Mastectomy	105	50.2
Widowed	12	5.9	Lumpectomy	97	46.4
Divorced	17	8.3	Other	7	3.3
Single	15	7.3	<b>Tumor size</b>		
Partner, not living together	7	3.4	<2 cm	87	44.4
<b>Living with</b>			2-5 cm	72	36.7
Partner	151	73.7	>5 cm	37	18.9
Other	2	1.0	<b>Type of cancer</b>		
Children	12	5.8	Ductal	150	73.2
Living alone	40	19.6	Lobular	34	16.6
<b>Education</b>			Other	21	10.2
Primary school	9	4.4	<b>Receptor</b>		
Secondary school	47	23.0	ER+/PgR+	134	72.0
Lower additional	26	12.7	ER+/PgR-	21	11.3
Higher additional	28	13.7	ER-/PgR+	13	7.0
University	95	46.3	ER-/PgR-	18	9.7
<b>Children</b>			<b>Treatment Postop</b>		
Yes	184	89.8	Chemotherapy (CT)	107	52.2
No	21	10.2	Radiotherapy (RT)	126	61.5
<b>Employment status</b>			Hormonal therapy (HT)	135	65.9
Working	133	68.2			
Unemployed	1	0.5			
Disability pensioner	8	4.1			
Retired	52	26.7			
Other	1	0.5			

Some of the variables deal with missing data

### IL-6

Mean serum concentration of IL-6 when all samples were taken together was 2.24 pg/mL (SD 5.70). When the group was subdivided in patients with early stage breast cancer and patients with recurrence there was a significant difference in concentrations of IL-6 ( $P < 0.001$ ). Mean value in the early stage breast cancer patients was 1.53 pg/mL (SD 1.3,  $N = 296$ ) and in the group with women with recurrence the mean value was 5.58 pg/mL (SD 12.9,  $N = 63$ ), illustrated in figure 1. There were

two very high values of IL-6 concentrations in the recurrence group, these are not seen in the figure to make it more visually clear.

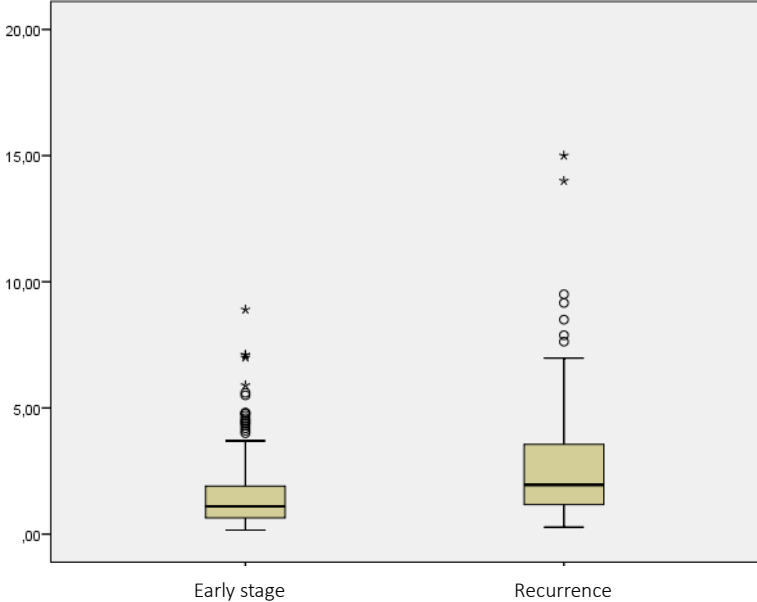


Figure 1. IL-6 levels in early stage breast cancer patients and patients with recurrence.

## Psychological outcomes

Table 2. Psychological outcomes

<i>Outcome</i>	<i>Mean value</i>	<i>SD</i>	<i>N</i>
<b>HAD</b>			
<i>Depression</i>	3.75	3.51	384
<i>Anxiety</i>	5.86	4.07	384
<b>MSAS</b>			
<i>Psychological</i>	1.12	0.84	401
<i>Physiological</i>	0.61	0.48	401
<i>Global Distress Index</i>	1.76	0.76	401
<i>Total MSAS</i>	0.68	0.44	401
<b>Coping capacity</b>	68.3	12.9	384
<b>PTGI</b>			
<i>Total Score</i>	57.6	19.6	370
<i>Relating to Others</i>	20.7	7.64	375
<i>New Possibilities</i>	12.2	5.43	376
<i>Personal Strength</i>	11.9	4.35	375
<i>Spiritual Change</i>	3.07	2.90	376
<i>Appreciation of Life</i>	9.86	3.02	375

## Correlations

Table 3. Correlations between IL-6 and the outcomes of the study

<i>Outcome</i>	<i>Correlation Coefficient</i>	<i>P-value</i>	<i>N</i>
<b>HAD</b>			
<i>Depression</i>	-0.044	0.411	350
<i>Anxiety</i>	-0.048	0.375	350
<b>MSAS</b>			
<i>Psychological</i>	-0.013	0.802	359
<i>Physiological</i>	0.173**	0.001	359
<i>Global Distress Index</i>	0.073	0.168	359
<i>Total MSAS</i>	0.104*	0.050	359
<i>Number of Symptoms</i>	0.127*	0.016	359
<b>Coping capacity</b>	0.086	0.110	350
<b>PTGI</b>			
<i>Total Score</i>	0.041	0.449	338
<i>Relating to Others</i>	0.029	0.596	343
<i>New Possibilities</i>	-0.043	0.430	344
<i>Personal Strength</i>	0.078	0.150	343
<i>Spiritual Change</i>	-0.034	0.526	344
<i>Appreciation of Life</i>	0.133*	0.014	343

\*correlation is significant at the 0.05-level (2-tailed), \*\*correlation is significant at the 0.01-level (2-tailed)

There were significant correlations between IL-6 levels and physical symptoms measured with MSAS, which was mirrored in the total symptom burden and number of symptoms as well; results are presented in table 3.

We also found a significant positive correlation between IL-6 and “Appreciation of Life”, a sub measurement of PTGI, with a correlation coefficient of 0.133 ( $P = 0.014$ ). The other items regarding psychological symptoms gave no significant correlations, presented in table 3. Subanalyses were done by dividing the population in patients with early stage breast cancer and patients with recurrence, then the correlation between IL-6 and “Appreciation for Life” was stronger in the early stage group (correlation coefficient 0.159,  $P=0.008$ ,  $N=281$ ) but it was non-significant in the recurrence group. A positive correlation was also found between coping capacity and IL-6 in the group with early stage breast cancer (correlation coefficient 0.127,  $P=0.031$ ,  $N=288$ ).

When subanalyzing the data in groups divided by recurrence or not, the correlation between IL-6 and physical symptoms is significant on  $p<0.001$  level in the group with patients with recurrence (correlation coefficient 0.433,  $P=0.000$ ,  $N=63$ ). However, there was no significant correlation between physical symptom, total symptom burden or number of symptoms in the early stage group in the subanalysis. The other subanalyses did not show any significant correlations, including analysis on HAD-score and IL-6.

## Discussion

### ***IL-6, physiological symptoms and disease stage***

Our main result regarding the IL-6 concentrations is that it is significantly higher in the group of women with a recurrence in breast cancer. Hence, our data support the idea of a correlation between systemic concentrations of IL-6 and a more advanced breast cancer stage (Ma et al., 2017). And as Ma et al has suggested, this correlation could be used to detect patients who could be helped from a more aggressive therapy. Our findings can be suggested to be in line with the recent proposition that IL-6 levels negatively correlate to overall survival in cancer. However, this correlation has only been shown in later stages of cancer in earlier studies (Lippitz & Harris, 2016).

We also found a positive correlation between IL-6 and physiological symptoms in later stages of breast cancer. However, there was no correlation between IL-6 and physiological symptoms in the group of women with early stage breast cancer.

### ***IL-6 and psychological symptoms***

In contrast to earlier studies (Seruga, Zhang, Bernstein, & Tannock, 2008) we did not find a correlation between IL-6 and cognitive symptoms in our population of patients with cancer. We did not find any correlation between IL-6 and HAD-score neither which has been proposed by recent research in patients with colorectal cancer (Miranda et al., 2017). On the other hand, our population had lower mean scores on HAD, patients diagnosed with MDD were excluded and the women in our study were going through a MBSR-intervention designed to help the women with symptoms of mood disorder. This might be part of an explanation to the fact that our result differs from earlier. Another explanation to the fact that our findings do not agree with earlier might be that our serum levels of IL-6 were lower than the median levels of cancer patients in other studies, the median of serum level in cancer patients have been reported as 6.95 pg/mL (Lippitz & Harris, 2016) and our median was 2.24 pg/mL.

Interestingly, the correlation between IL-6 and coping capacity and Appreciation for Life in this study, was positive. It has been suggested that measuring IL-6 without measuring sIL-6R cannot

give any information on the inflammatory consequences (Maes et al., 2016) and the effects of IL-6 on tumor growth has not been agreed upon (Dethlefsen et al., 2013). Thus, it may seem fruitless to argue whether a positive or negative correlation has any transferability to good or bad in sense of inflammatory response or cancer progress. Finding correlations or not is how we move our knowledge forward and it seems as we might have come to an edge on the research of how IL-6 on its own relates to inflammation, but like all edges it is just the beginning of something new. Arguing around our findings one could say that because coping capacity is associated with higher resilience to stress it would logically then be negatively correlated to IL-6, since IL-6 is positively correlated to depression. High responses in IL-6 concentration to stress is correlated to a higher vulnerability to stress (Menard, Pfau, Hodes, & Russo, 2017). Therefore, our results with a positive correlation is intriguing, and to our knowledge no one has done this testing before and it would be interesting to see if it is reproducible.

### ***The way forward for inflammation-depression research***

The growing elderly population and improvements in diagnosis and treatment of cancer is resulting in a growing population of cancer survivors. This also means that we must deal with more of the physical and affective sequelae that often follows cancer diagnosis and treatment. Many cancer survivors develop depression, cognitive disturbances, fatigue and sleep disturbances that cannot be explained merely by the medical complications of the treatment (Miller et al., 2008). A better understanding of the factors affecting the comorbidity in this growing population is highly wanted. Psychoneuroimmunology is emerging as a field with possible answers.

Since 1995 when Michael Maes suggested the inflammatory hypothesis in depression it has been the golden standard to measure IL-6 levels as a sign for immune response in patients with mood disorders (Maes, 1995; Musselman et al., 2001), but now evidence is emerging pointing away from looking at only peripherally IL-6, there is a need for seeing the bigger picture when doing research on the immune system and its effects on mood (Maes et al., 2016). The immune system is complex,

and we are in a process of learning more on the different pathways. The results have been varying on the IL-6 correlation to symptoms of depression in cancer patients, however IL-6 is significantly elevated in populations of patients diagnosed with MDD, with or without co-morbidity (Miller et al., 2008).

Hodes et al has been refining the research on how to integrate IL-6 levels into depression diagnosis and treatment, by looking at an individual's peripheral acute IL-6 response to a stimulus. A high response in rodents have been correlated with higher susceptibility to development of depression-like behavior when the rodent is being placed in a situation of social stress or by merely witnessing social stress (Hodes et al., 2014). She has also suggested different pathways for IL-6 signaling in CNS vs peripherally (Hodes et al., 2016). This is suggesting we could focus more on measuring an individual's IL-6 response to acute stress to predict the psychological burden outcome of chronic stress, which living with a cancer diagnosis could be, as an alternative to other suggestions of measuring IL-6 and behavior longitudinally to adjust for the fluctuating IL-6 levels over short time (Miller et al., 2008).

Taking the complexity of the immune system into account, the way forward for depression-inflammation research, with or without comorbidity, should be omics-based and use systems biomedicine methodologies to present new possible solutions for prevention and treatment (Maes et al., 2016).

### ***Limitations***

One of the limitations of our study is that the data comes from two observations with the same individuals, this may compromise the variation of the IL-6 levels. Other limitations of our study is that Body Mass Index (BMI) also affects the IL-6 levels (Felger & Lotrich, 2013) and when doing the statistical analysis we did not have this information. However, one recent study showed that there were no significant differences in serum IL-6 in breast cancer survivors divided by normal weight, over weight and with obesity (Babaei et al., 2015). We also know that physical activity



affect IL-6 (Gleeson et al., 2011) and we cannot adjust for this since we do not have any records of the subject's physical activity. Also, infections do increase the IL-6 concentrations, there is no excluding of subjects having a minor temporary infection leaving blood samples. Some of the physiological symptoms correlating to IL-6 concentrations in our study (cough, nausea, breathlessness, difficulty swallowing, diarrhea and loss of appetite) could be signs of ongoing infection, thus the infection would be a confounding factor not adjusted for. Our study is also limited by the fact that we could not adjust for diet. Diet affects IL-6, one example is that high-fat meals increase the concentrations and a Mediterranean diet have been shown to decrease IL-6 concentrations (Maggio et al., 2006). Our study was also limited because it did not exclude patients receiving hormonal therapy, and this can also interfere with IL-6 production (Koka, Petro, & Reinhardt, 1998). One could in future analysis do sub analysis dividing the women in groups of receiving or not receiving hormonal therapy. Lastly, we do not know if the method measures IL-6 only or if it also detects IL-6 when bound to sIL-6R and sIL-6R/gp130 and we did not measure sIL-6R or sGP130, hence we do not know if the anti-inflammatory pathway is upregulated or if the transcription is inhibited in a higher or lower proportion.

Some of the strengths in our study was the large population and that none of the participants had ongoing chemotherapy nor trastuzumab (Herceptin)

therapy. Chemotherapy can affect IL-6 levels in cancer patients (Jardim Paz et al., 2017) and Herceptin was excluded because it was expected to effect the immune system. Also, some chronic diseases (Type 1 diabetes, Crohn's disease, Rheumatoid Arthritis) may affect the IL-6 concentrations (Maggio et al., 2006), patients diagnosed with these diseases were excluded since they counted as having another advanced illness.

## Conclusion

There is a need for finding better tools for an earlier discovery of recurring breast cancer. Our results support the idea that systemic IL-6 is positively correlated to disease stage in breast cancer patients, which could be regarded as in line with the recent proposal that IL-6, in late stage cancer, could be a possible prognostic marker for overall survival in cancer patients and a marker for a more advanced disease.

More and more women are surviving primary breast cancer, but they suffer from sequelae, particularly affective symptoms. We need to learn more on how to best care for this group after the diagnosis and treatment. Psychoneuroimmunology and breast cancer is an emerging area of interest. Research has suggested that IL-6 concentrations positively correlates with symptoms of mood disorder in oncology patients, however in this study we did not find this correlation in our population of women with breast cancer, patients with ongoing MDD was excluded. A more refined understanding of how to best analyze the interplay between our immune system and our behavior and mood in cancer patients is desirable.

We could find positive correlations between IL-6 and coping capacity, and IL-6 and personal growth. These results seem inconclusive, and further strengthens the modern idea of a more omics-based future research where the use of systems biomedicine methodologies could be favorable to understand more about our immune system's involvement in psychological resilience and susceptibility to stress.

## Populärvetenskaplig sammanfattning

Efter en cancerdiagnos är det vanligt att drabbas av depression. Symtomen för depression är dock samma som många av bieffekterna av cancerbehandlingen vilket gör att det är svårt att veta vilka av patienterna som behöver hjälp för sina depressiva besvär och vilka som självmant kommer att bli bättre efter behandlingen. De senaste decennierna har det forskats på länken mellan vårt immunförsvar och depression och man har funnit att cytokinen IL-6 (Interleukin 6) är förhöjd vid depression hos cancerpatienter jämfört med patienter som har cancer men inte har en klinisk depression. Denna studie har undersökt sambandet mellan IL-6 i blodet och psykologiska och fysiologiska symtom hos 208 patienter med bröstcancer uppdelat i tidigt och sent stadie. Kvinnorna som hade tidigt stadie av bröstcancer hade mycket lägre IL-6 än kvinnorna som hade återfall i bröstcancer. Tidigare har forskare visat att ett högre IL-6 är sämre för den generella prognosen vid cancersjukdom.

Vi hittade en tydlig positiv korrelation mellan IL-6 och fysiologiska symtom hos gruppen med återfall. Kvinnorna med tidigt stadie hade lägre IL-6 nivåer än vad patienter med cancer har haft i andra studier men här fann vi en positiv korrelation mellan IL-6 och känsla av sammanhang samt mot uppskattning av livet. Vi har inte hittat några tidigare studier som utforskat länken mellan liknande psykologiska utfall och immunsystemet. Resultatet är intressant och bör testas igen för att kunna dra några slutsatser från. Vi fann dock ingen korrelation mellan IL-6 och depressiva symtom eller ångest i någon av grupperna, dock hade våra patienter både lägra median IL-6 och mindre depressiva besvär än vad populationerna i tidigare studier har haft.

Forskningsfältet inom immunologi har varit aktivt den senaste tiden och nya upptäckter har gjorts. Vi vet nu att IL-6 är mer komplex än vad vi trodde från början, för beroende på vart receptorn som binder IL-6 sitter så kommer den att ge en signal som är antingen inflammatorisk eller anti-inflammatorisk. Detta gör att det är svårt att dra slutsatser om de immunologiska effekterna av IL-6

men man kanske kan använda våra resultat för att tidigare hitta patienter med återfall eller se ett förhöjt IL-6 som en biomarkör för en svårare sjukdom.

Att det finns en länk mellan vårt immunsystem och våra beteenden och humör står klart, det forskas på detta under benämningen psykoneuroimmunologi. Den framtida forskningen kräver mer avancerade metoder för att kunna titta på fler nivåer av immunsvaret så att man kan se vilka gener som finns, vilka som uttrycks, vilka av dessa som faktiskt blir till funktionella proteiner och även hur dessa sedan metaboliseras i kroppen.

Kvinnor som lever med en bröstcancerdiagnos blir fler och fler då vi har bättre metoder för att upptäcka och behandla. Bröstcancer kan idag ses mer som en kronisk sjukdom och vi behöver lyfta forskningen till hur vi bäst tar hand om den växande gruppen av överlevare och hur vi snabbt hittar de som får återfall.

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