# The Association between Psychosocial Factors and Pro-Inflammatory Cytokines (IL-6 and IL-8) in Colorectal Cancer Patients

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**Abstract**

*Background:* Colorectal cancer (CRC) is the third most common cancer among both men and women, and progression of CRC has been linked to pro-inflammatory states. Psychological factors, such as loneliness and poor social support, have been associated with increased serum levels of pro-inflammatory cytokines in a variety cancer cell types.

*Methods:* As part of the Can Support pilot study, participants with newly diagnosed CRC provided blood samples and completed the PROMIS short form survey measures for social isolation, emotional support, companionship, informational support, and instrumental support prior to undergoing surgery. Serum IL-6 and IL-8 levels were determined via ELISA. Correlation as well as simple and multiple linear regression were used to assess the relationship between the serum cytokine levels and psychosocial factors.

*Results:* From a total of 216 screened patients, complete data were obtained from 39 patients. The average age at diagnosis was 57.9 years, and 51.3% were women. Due to protocol deviations, a total of 6 patients had blood drawn while in the OR as opposed to during standard pre-operative office visits. Average IL-6 values trended towards significantly differing with respect to draw-time ($p < 0.087$). None of the five psychosocial variables correlated with normalized values of serum IL-6 or IL-8 values. Simple linear regression models were created for each psychosocial and bio-marker pair, and none of the psychosocial variables significantly explained or correlated with either IL-6 or IL-8. Multivariate analysis with relevant covariates was completed for each psychosocial and bio-marker pair. Only instrumental support was identified as an independent predictor of IL-8 serum levels when adjusting for various covariates.

*Conclusion:* There was little to no observed association between various social support measures and serum IL-6 or IL-8 levels, but the results do help to highlight the importance of protocol design, namely blood collection time, which helps to inform future trial design.

**Background and Statement of Public Health Relevance**

Colorectal cancer (CRC) is the third most common newly diagnosed cancer in both men and women as well as the third most deadly for each gender, accounting for over 50,000 deaths in the United States in 2015 (1). Current preventative strategies include the use of colonoscopy and stool-based tests for blood and DNA (2). However, screening is generally thought to be invasive, expensive, and time-consuming leading to a relatively low adherence to screening guidelines. In fact, one retrospective study observed only a 64% adherence rate to United States Preventative Service Task Force screening guidelines in a population of over 150,000 insured adults between the ages of 50 and 60 years old (3). Therefore, CRC is still diagnosed at relatively high rates: 38.0 new diagnoses per 100,000 people (age-adjusted). Unfortunately, metastatic/distant disease continues to be deadly with a relative 5-year survival rate of only 12-14% (1, 4). There is a sustained need to understand the development and progression of CRC within the United States as well as to develop comprehensive public health strategies for the primary and secondary prevention of colorectal cancer.

Chronic inflammation is a known driver of both CRC development and progression. Inflammatory disease states, such as ulcerative colitis (UC), are clearly linked to an increased risk of CRC, with UC patients having an 18% chance of developing CRC within thirty years from disease onset (5). Inflammation is also thought to promote the progression of established

tumors of various origins via the development of new blood vessels, which serve to nourish tumors, and by the inhibition of innate immunity, allowing unchecked tumor cell proliferation (6).

During inflammatory states, the body upregulates a variety of cytokines (chemical mediators). These cytokines then work to augment the immune system and, as a result, can provide either a favorable or toxic environment for the development of malignant cells. While several cytokines are up-regulated within colorectal cancer patients, interleukin-8 (IL-8) is one of the most significantly overexpressed. IL-8 is involved in a variety of normal physiologic signaling pathways. However, it can directly interact with signaling pathways within CRC cell lines promoting unchecked cell proliferation as well as migration/ metastasis (6 - 9). Similarly, interleukin-6 (IL-6) levels are significantly increased in patients with CRC compared to healthy controls and are correlated with tumor size and metastatic disease (10, 11). Although inflammation is a key risk factor for cancer development and progression as evidenced by the increased risk of cancer development in UC patients, more knowledge is needed to understand the key drivers of inflammation and elevated pro-inflammatory cytokines in patients without these specific inflammatory disease states.

As previously described, inflammatory bowel disease can drive localized inflammation, but other, less obvious drivers of inflammation, such as adverse psychosocial factors (e.g. lack of social support), are also linked to pro-inflammatory states. Adverse psychosocial situations are associated with increased levels of pro-inflammatory and pro-angiogenic cytokines in both cancer cell lines and newly diagnosed cancer patients' serum (12 – 15). The current causal model based on recent evidence suggests that elevated psychological stress leads to the

upregulation of the hypothalamic-pituitary axis. Then, through various endocrine signaling pathways, norepinephrine (NE) is released which further induces the expression of pro-inflammatory cytokines including VEGF, IL-6 and IL-8 (13). This relationship between NE and VEGF, IL-6, and IL-8 has been directly observed in melanoma cell lines (16). Thus, psychosocial factors are associated with an increased angiogenic state via VEGF and a compromised innate immune system via dysregulation of IL-6 and IL-8 signaling.

A variety of studies have linked both depressive symptoms and social isolation/support with varying levels of VEGF and IL-6. Within CRC patients, increased loneliness has been associated with significantly elevated histochemical tumor levels of VEGF but not with elevated levels of IL-6 (12). This provides a potential link between loneliness and, by extension, social isolation and the development/ progression of CRC, presumably mediated by the release of NE. Other studies have linked increased serum VEGF levels and depression within patients with CRC (14) and decreased serum VEGF levels with increased social support in ovarian cancer patients (15). With regards to IL-6, social attachment was associated with lower serum IL-6 levels while greater vegetative depression was linked to elevated serum levels (15, 17). However, an investigation of the relationship between social support and serum IL-6 and IL-8 levels has yet to be explored within colorectal cancer patients.

Characterizing the impact of psychosocial conditions in CRC can lead to greater understanding of CRC cancer development and progression and serve as the basis for improved cancer prevention programs as well as patient support and treatment programs. The link between psychosocial factors and pro-inflammatory bio-markers has implications for public health. Taking Chicago as an example, the Healthy Chicago 2.0 initiative included the goal of

"reduc[ing] the incidence of and inequities in invasive and late stage cancer and cancer mortality." (18) While age-appropriate cancer screening – one of the key objectives of Healthy Chicago 2.0 - is an important part of this goal, recognizing the links between other factors such as social support and the theoretical risk of cancer progression may identify new targets for community-based interventions.

## Materials and Methods

### *Initial Patient Recruitment and Enrollment*

Patients meeting the inclusion criteria for the Can Support Pilot Study were screened for participation in the study. The inclusion criteria were ≥ 18 years old, histologically confirmed colorectal cancer with a clinical stage I – IIIC by imaging, scheduled for tumor resection within one month, and the ability to read, write, and understand English. Patients with stage IV disease based on imaging, those with conditions affecting the immune system (e.g., celiac disease, Crohn's disease, multiple sclerosis, inflammatory bowel disease, ulcerative colitis, rheumatoid arthritis or lupus), and those receiving corticosteroids a month prior to blood draw were excluded from the study. A total of 216 patients at Northwestern Medicine Clinics were screened for eligibility. Of those screened, 130 were deemed ineligible or declined to participate. An additional 40 participants were missed, most often due the patient missing the pre-operation appointment. A total of 46 patients enrolled in the study. However, two patients became ineligible. Two patients withdrew from the study. Two patients did not provide sufficient blood for analysis, and one patient was lost to follow-up. Thirty-nine participants provided both survey answers and sufficient blood for analysis. However, some patients did not

complete all survey questions, thus limiting certain analyses. This study was approved by Northwestern University institutional review board.

*Initial Data Collection*

Patients were initially approached during pre-operative appointments (within one month of surgery) within a colorectal surgical oncology clinic associated with a large academic medical center in Chicago, IL. Trained phlebotomists collected patient consent and peripheral blood samples at a designated clinic appointment prior to surgery. However, six patients provided blood samples on the day of surgery in the operating room (OR), often after the initiation of anesthesia.

Patient demographic and clinical information was collected in two forms. Study staff collected clinical patient characteristics via medical record or electronic health record (EHR) query. Demographic information and psychosocial measures were collected via patient-report in AssessmentCenter$^{SM}$ within one week of their blood draw (19). Following surgery, medical staff obtained the patient's pathological stage via EHR query.

*Psychosocial Measures*

Participants completed the Patient-Reported Outcomes Measurement Information System (PROMIS) v2.0 Short Forms, which have been previously validated (20). The short forms used for analysis included Social Isolation 6a, Emotional Support 4a, Companionship 4a, Informational Support 4a, and Instrumental Support 4a. Each short form consists of 4-6 questions. Scores were scaled and normalized per the PROMIS Scoring Manual (21). For the

social isolation, emotional support, companionship, and instrumental support forms, all 39 participants completed all questions needed for analysis. However, for the informational support form, only 38 participants completed enough questions needed for analysis.

*Potential Covariates*

As stated previously, patients answered questions related to several demographic, clinical, and lifestyle factors. Personal characteristics included gender, age (categorized by age at diagnosis), race, ethnicity, BMI (calculated and categorized), insurance status, marital status, employment status, and income. Oncologic variables included T/M/N staging (independently reported), pathologic grade, KRAS mutation status, and BRAF mutation status. With regards to current medication use, patients reported taking beta-blockers, anti-depressants, aspirin, or statins. Lifestyle metrics included frequency of non-steroidal anti-inflammatory drug (NSAID) use, physical activity, carbohydrate intake, fruit/vegetable intake, processed and red meat intake, smoking, alcohol use, as well as relaxation and stress relief activities. Finally, patients were asked to report a variety of comorbidities including hypertension, coronary artery disease, congestive heart failure, prior transient ischemic attack (TIA), liver disease, rheumatoid arthritis, osteoarthritis, migraines, asthma, chronic obstructive pulmonary disease (COPD), diabetes, depression, anxiety, sleep disorders, HIV/AIDS, spinal cord injuries, or multiple sclerosis. All demographic, oncologic, drug, and lifestyle related variables were assessed as possible covariates, further described in the statistical analysis below.

A few variables were calculated from the provided information prior to the covariate analysis. A raw comorbidity count was generated for each patient which was then used as a

clinical variable within the analysis (as opposed to each individual comorbidity). TMN clinical stage (0-III) was also calculated based on the American Joint Commission for Cancer Staging Manual (22). Patients with known or later-identified metastases were excluded from the study. Therefore, for staging calculations, patients were presumed to have stage M0 disease (non-metastatic), and with this consideration, 35 patients had complete staging information. Finally, due to protocol deviations, a total of 6 patients had blood drawn while in the OR as opposed to during the standard pre-operative office visit. A bivariate draw time variable (blood drawn in OR vs. drawn at pre-operative office visit) was included in the covariate analysis.

*Cytokine Measurement*

Cytokine levels were experimentally obtained by a collaborating lab core. Peripheral venous blood was assayed for IL-6 and IL-8 cytokines from serum using ELISA. Per laboratory documentation, one 8.5 mL tube of venous blood in an evacuated tube with spray-coated silica and a polymer gel for serum separation was obtained. Evaluation of cytokines in serum was performed using the commercial ultrasensitive solid phase ELISA kits: Invitrogen ELISA kit KHC0062 for Human IL-6 (Sensitivity: <2 pg/ml, Concentration: 7.8 – 500 pg/ml) and Invitrogen ELISA kit KHC0082 for Human IL-8 (Sensitivity: <5 pg/ml, Concentration: 15.6 – 1000 pg/ml). Serum cytokine levels were used for analysis.

*Statistical Analysis*

Data were analyzed using SAS University Edition; SAS version 15.1 (23). Descriptive statistics (average, standard deviation, ranges, etc.) were generated for demographic and

clinical patient data. Cytokine levels were assessed for normality, and logarithmic transformation for IL-6 and IL-8 serum levels was preformed prior to analysis.

Initial differences in serum IL-6 and IL-8 levels with respect to categorical patient demographic and clinical variable (age category, stage, etc.) were assessed using one-way ANOVA. Similarly, differences in psychosocial variable scores with respect to patient demographic and clinical variables was assessed using one-way ANOVA. Demographic or clinical variables significant to both a bio-marker and psychosocial variable at $p < 0.20$ were included as covariates in the multiple linear regression model for said bio-marker, psychosocial variable pair (24).

Spearman correlations were performed to identify initial, unadjusted, associations between psychosocial measures (Social Isolation, Emotional Support, Companionship, Informational Support, and Instrumental Support) and biomarkers (IL-6 and IL-8). The impact of psychosocial variables on IL-6 and IL-8 serum levels was then assessed using a series of linear regression models. Univariate linear regression models were created for each psychosocial and biomarker pair (Normalized BioMarker Level = PROMIS Short Form Calculated Score + Error). Multiple linear regression was then performed by adding clinically relevant covariates and covariates related to both bio-marker and psychosocial variable (as defined above). Stage was deemed clinically relevant for all modes (10, 11) and draw time was deemed relevant for all models involving the association between psychosocial variables and IL-6 levels. Of note, covariates in the final multiple regression models were limited to a total of four due to small sample size and to minimize the effect of overfitting. In the case that more than four covariates were clinically and/or statistically relevant, variables were chosen in a stepwise manner. This

was achieved by adding all relevant variables to the model initially, and then, with each iteration of the fully adjusted model, the least significantly related variable was dropped from the model until only four covariates remained.

## Results

*Patient Details*

Complete demographic data was obtained from 39 patients with newly diagnosed CRC requiring surgery. The demographic and relevant oncologic characteristics are displayed in Table 1. The average age at diagnosis was 57.9 years (standard deviation 12.0 years, range 30-82 years), and 51.3% were women. Most patients self-described themselves as White/Caucasian (89.7%). Participants were generally well-educated, with over 97% having at least some college education. With regards to clinical stage, one patient (2.56%) had Stage 0 disease while 10 (25.64%) had Stage I, 10 (25.64%) had Stage II, 14 (35.90%) had Stage III disease, and 4 individuals (10.26%) had incomplete information for staging calculation. Further, five patients (14.3%) had a known KRAS mutation while zero patients had a known BRAF mutation. Additional lifestyle, medication, and comorbidity information was collected and included in potential cofounder discovery analysis (details below). Of note, 17 patients (43.6%) did not self-report any comorbidities. Eleven patients (28.2%) identified one comorbidity, and the remaining eleven patients (28.2%) identified more than one (max of seven) comorbidities. The mean raw serum IL-6 level was 1.81 pg/mL (SD = 4.75 pg/mL; range 0.27 - 29.67 pg/mL), and the mean raw serum IL-8 level was 14.76 pg/mL (SD = 7.03 pg/mL; range 5.36 - 41.16 pg/mL).

*Relationship between psychosocial variables, bio-markers values, and potential confounders*

All clinical and demographic variables were assessed as potential confounders. IL-6 values were significantly related to marital status, employment status, BMI category, and frequency of physical activity (at $p < 0.20$; Table 2). IL-8 values differed with respect to age category, gender, education, employment status, annual income, anti-depressant use, fruit and vegetable intake, and smoking status (at $p < 0.20$; Table 2). Of note, several of the variables associated with the bio-marker levels, had categories including only one patient (e.g. only one participant had a given level of "$20,000 - $24,999" annual income). Given the relatively small sample size and the desire to avoid biased selection of covariates, the variables continued to be included in the analysis with the same pre-specified categories even if only one participant fell into a given category. Importantly, neither IL-6 nor IL-8 levels were associated with stage. Stage was included in the fully adjusted linear regression models for both bio-markers due to clinical importance and previous work showing the relationship between disease stage and cytokine levels (10, 11).

A similar analysis was undertaken for the relationships between the psychosocial variables and potential cofounders (Table 3). Each psychosocial variable was associated with a variety of demographic and clinical variables. Variables significant to both bio-marker and psychosocial variable at a level of $p < 0.20$ were included in the fully adjusted multiple linear regression model.

Average serum IL-6 values trended towards significantly differing with respect to draw-time ($p < 0.087$; Figure 1). Those with blood draw in the OR tended to have higher serum levels

of IL-6 when compared with those who had their blood drawn at the pre-operative visit. Average IL-8 serum levels appeared unrelated to draw-time ($p < 0.4783$). A binary variable representing blood draw time was included in the multivariate models assessing the response of IL-6 levels.

*Association between serum cytokine levels (IL-6 and IL-8) and psychosocial variables*

None of the five psychosocial variables (social isolation, companionship, informational support, instructional support, or emotional support) correlated via Spearman correlation with log-transformed (normalized) values of serum IL-6 or IL-8 values (Table 4). Informational support's negative correlation with IL-6 levels did trend towards significance ($r = -0.30350$; $p < 0.064$).

*Simple linear regression between psychosocial variables and bio-marker variables*

Simple linear regression models were created for each psychosocial and bio-marker pair (Table 5). None of the psychosocial variables were significantly associated with either IL-6 or IL-8 serum levels. Of note, an inverse relationship between informational support and IL-6 was observed, but this relationship was not statistically significant (p-value = 0. 1479).

*Multiple linear regression between the independent psychosocial variables and the dependent IL-6 and IL-8 levels*

Multivariate analysis was completed for each psychosocial and bio-marker pair with the psychosocial variable modeled as a potential independent predictor. The covariates included in

each model can be found in Table 6. None of the five psychosocial variables were significant independent predictors of IL-6 serum levels. However, draw time was significantly associated with IL-6 in all five models. With regards to IL-8 response, instrumental response negatively predicted IL-8 values ($p < 0.01$) when adjusted for disease stage, age at diagnosis, and annual income. Social isolation, emotional support, and companionship were not significantly associated with IL-8.

**Discussion**

This work was a sub-analysis of data initially collected in the Can Support pilot study and investigated the relationship between PROMIS short form scores related to social support and serum IL-6 and IL-8 levels. The larger Can Support trial sought to: 1) investigate the feasibility of a bio-marker/ survey-based study and 2) explore the association between various patient reported health outcomes and cytokine levels. The results of this sub-study are overwhelmingly negative, showing little to no association between various social support measures and serum IL-6 or IL-8 levels. However, the results do support feasibility of recruiting patients recently diagnosed with CRC in local clinics, prior to surgery, and help to highlight the importance of protocol refinements for future trial design.

As stated, no association was observed between any of the PROMIS short form scores and serum IL-6 values neither in the correlation nor the regression analysis. A prior study completed by Nauseen et al. found that loneliness (which may serve as a proxy for poor social support) was not associated with IL-6 histochemical expression which is consistent with our negative findings (12). Work completed by Ashizawa et al. and Chung et al. noted IL-6 levels were

significantly related to CRC stage with advanced stages having higher levels of circulating cytokine (10, 11). Further, only levels > 25.0 pg/mL were associated with immunohistochemical staining in the actual malignant cells (11). Interestingly, in our study, IL-6 values did not significantly differ with respect to stage, likely since metastatic disease was excluded from study. Of note, approximately 53% of our participants had earlier disease stages (stage 0-II). Perhaps, psychosocial factors do not exert a toxic (or protective) effect during earlier stages of disease (lower circulating serum IL-6). This hypothesis is supported by the observation that stimulated IL-6 expression is not correlated with perceived social support in a relatively healthy middle-aged community population when controlling for demographic factors (25). While the experimental process for obtaining IL-6 levels was different and must be interpreted carefully, the general dissociation between social support and the potential for IL-6 cytokine production still holds true. Conversely, Costanzo et al.'s research which did associate IL-6 serum levels with a wide variety of psychosocial factors was primarily conducted in advanced stage ovarian cancer (15). Overall, IL-6 serum levels do not appear to be related to social support factors, particularly in earlier disease stages.

No study to date, to the best of our knowledge, has investigated the association between serum IL-8 levels and social support within cancer patients. Our findings suggest that IL-8 is largely unrelated to various measures of social support. However, instrumental support (having individuals in one's life to help with physical activities) was a significant negative independent predictor of serum IL-8 levels when adjusting for stage, age, and annual income. This may suggest some relationship between social support and IL-8, but looking at the global trends of the results, the associations between the PROMIS short form scores and IL-8 is inconsistent and

largely insignificant. Interestingly, one study did observe that higher levels of social support were associated with lower levels of stimulated production of IL-8 in a relatively healthy middle-aged community population (25). This may indicate that IL-8 is in fact related to social support but serum measurement of IL-8, a marker of systemic inflammation, is not specific enough to delineate a consistent relationship as compared to directly stimulated cells.

A major limitation of this study is that 15.3% of participants had a significant protocol deviation by having their blood drawn while in the OR. Draw time was consistently identified as a significant independent predictor of serum IL-6 levels when adjusting for a variety of other covariates. The timing of the blood draw seemed to disproportionately affect IL-6 levels compared to IL-8 levels. This may indicate that a physical or social stressor (i.e. major abdominal surgery) can quickly alter the systemic levels of cytokines. Further, it is unclear if some of these participants had their blood drawn after the initiation of surgery as surgical trauma has clearly been linked to elevated levels of IL-6 and IL-8 (26). It is somewhat puzzling that IL-8 was not similarly increased in our results. It should also be noted that a large meta-analysis found that IL-6 levels vary throughout the day with a trough in the morning around 8:00-9:00AM in health adults (27). This further supports the premise that consistent time and location of blood draws is needed for accurate analysis.

Another limitation of this study is its relatively small sample size and the inherent impact of a recent cancer diagnosis. Numerous psychosocial factors were assessed along with a variety of demographic and clinical variables. For the number or patient-reported outcomes and dependent cytokine variables, a larger sample size may have helped to uncover more subtle differences in cytokine variability. Given this study was conducted within a newly diagnosed

cancer population, sub-acute variations in social support measures (recent change in perceived amount of support) may have affected the results.

This study is a cross sectional correlational analysis in a relatively narrow patient population. The patients were fairly homogeneous with regards to race and socioeconomic background, and the results might not be applicable to a wider audience. Causal relationships cannot be implied from this study. Given available laboratory and observational data it is theorized that higher circulating levels of pro-inflammatory cytokines are a negative prognostic indicator. However, further longitudinal studies linking psychosocial factors, serum bio-markers, and cancer-related outcomes are needed.

**Conclusion**

Our results did not evidence significant relationships between serum IL-6 levels and various patient-reported social support metrics. However, the timing of blood draw did seem to impact serum IL-6 levels with those having their blood drawn in the OR having higher levels of circulating cytokine. Similarly, no psychosocial factors were significantly correlated with IL-8 serum levels. Only instrumental support was identified as an independent predictor of IL-8 serum levels when adjusting for various covariates. Results of this small pilot study indicated little to no relationship between the PROMIS short form social support measures and IL-6 or IL-8 serum values in patients with newly diagnosed CRC.

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Table 1. (A) Summary of patient demographic variables (B) Summary of patient clinical variables

*Stage was calculated for those with complete staging data.

(A)

| | N | (%) |
|---|---|---|
| ***Age Category*** | | |
| *Less than 40 years old* | 2 | (5.1) |
| *40-49 years old* | 8 | (20.5) |
| *50-59 years old* | 10 | (25.6) |
| *60 years old or older* | 19 | (48.7) |
| ***Gender*** | | |
| *Male* | 19 | (48.7) |
| *Female* | 20 | (51.3) |
| ***Ethnicity*** | | |
| *Non-Hispanic* | 38 | (97.4) |
| *Hispanic, Latino/a, Spanish Origin* | 1 | (2.6) |
| ***Race*** | | |
| *White/Caucasian* | 35 | (89.7) |
| *Black/ African American* | 2 | (5.1) |
| *Asian* | 2 | (5.1) |
| ***Marital Status*** | | |
| *Single* | 1 | (2.6) |
| *Married* | 33 | (84.6) |
| *Living with partner in committed relationship* | 1 | (2.6) |
| *Divorced* | 2 | (5.1) |
| *Widowed* | 2 | (5.1) |
| ***Highest Level of Education*** | | |
| *Some high school* | 1 | (2.6) |
| *Some college / Technical or Associate degree* | 7 | (17.9) |
| *College* | 13 | (33.3) |
| *Some graduate school* | 5 | (12.8) |
| *Graduate school / Advanced degree* | 13 | (33.3) |
| ***Employment status*** | | |
| *Full-time employed* | 19 | (48.7) |
| *Part-time employed* | 6 | (15.4) |
| *Retired* | 7 | (17.9) |
| *On disability* | 1 | (2.6) |
| *On leave of absence* | 1 | (2.6) |
| *Homemaker* | 4 | (10.3) |
| *Unknown* | 1 | (2.6) |
| ***Annual Income*** | | |
| *Less than $10,000* | 2 | (5.1) |
| *$20,000 - $24,999* | 1 | (2.6) |
| *$75,000 - $99,999* | 2 | (5.1) |
| *$100,000 - $124,999* | 3 | (7.7) |
| *$125,000 - $149,999* | 1 | (2.6) |
| *$150,000 - $199,999* | 8 | (20.5) |
| *$200,000 - $249,999* | 3 | (7.7) |
| *$250,000 or more* | 9 | (23.1) |
| *Don't know* | 2 | (5.1) |
| *Prefer not to answer* | 8 | (20.5) |
| ***Insurance Coverage*** | | |
| *No* | 1 | (2.6) |
| *Yes* | 37 | (94.9) |
| *Unknown* | 1 | (2.6) |

(B)

| | N | (%) |
|---|---|---|
| ***Pathologic Stage (T)*** | | |
| *T0* | 1 | (2.9) |
| *T1* | 5 | (14.3) |
| *T2* | 9 | (25.7) |
| *T3* | 17 | (48.6) |
| *T4a* | 2 | (5.7) |
| *T4b* | 1 | (2.9) |
| ***Pathologic Stage (N)*** | | |
| *N0* | 21 | (60.0) |
| *N1* | 10 | (28.6) |
| *N2* | 4 | (11.4) |
| ***Pathologic Stage (M)*** | | |
| *MX* | 20 | (57.1) |
| *M0* | 4 | (11.4) |
| *Unknown* | 11 | (31.4) |
| ***KRAS mutation*** | | |
| *No* | 9 | (25.7) |
| *Yes* | 5 | (14.3) |
| *Unknown* | 21 | (60.0) |
| ***BRAF mutation*** | | |
| *No* | 13 | (37.1) |
| *Unknown* | 22 | (62.9) |
| ***Pathological grade*** | | |
| *Grade 1* | 1 | (2.9) |
| *Grade 2* | 27 | (77.1) |
| *Grade 3* | 2 | (5.7) |
| *Unknown* | 5 | (14.3) |
| ***Stage**** | | |
| *Stage 0* | 1 | (2.6) |
| *Stage I* | 10 | (25.6) |
| *Stage II* | 10 | (25.6) |
| *Stage III* | 14 | (35.9) |
| *Unknown* | *4* | *(10.3)* |

Table 2. Association between cytokine levels with respect to select demographic variables as measured by ANOVA.

*ANOVA significant at P < 0.20

| | Normalized Serum IL-6 | | Normalized Serum IL-8 | |
|---|---|---|---|---|
| **Demographic Variable** | **F-Value** | **Pr > F** | **F-Value** | **Pr > F** |
| Age Category | 1.03 | 0.3915 | **1.77** | **0.1703*** |
| Gender | 0.06 | 0.8158 | **1.84** | **0.1833*** |
| Ethnicity | 0.09 | 0.7711 | 0.33 | 0.568 |
| Race | 0.41 | 0.669 | 0.83 | 0.4452 |
| Marital Status | **1.72** | **0.1673*** | 0.3 | 0.876 |
| Highest Level of Education | 0.21 | 0.9311 | **2.14** | **0.0971*** |
| Employment Status | **4.18** | **0.0033*** | **2.59** | **0.0371*** |
| Annual Income | 0.43 | 0.9083 | **2.7** | **0.0204*** |
| Insurance Coverage | 0.71 | 0.4965 | 0.14 | 0.8684 |
| KRAS Mutation | 0.07 | 0.9327 | 0.22 | 0.8054 |
| BRAF Mutation | 0.16 | 0.6954 | 0.53 | 0.4718 |
| Stage (derived) | 0.84 | 0.4812 | 1.22 | 0.3176 |
| Beta-Blocker Use | 0.03 | 0.867 | 0.54 | 0.4663 |
| Anti-Depressant Use | 0.38 | 0.5417 | **2.18** | **0.1479*** |
| Aspirin Use | 0.43 | 0.7357 | 0.66 | 0.5819 |
| Statin Use | 0.27 | 0.6073 | 0.13 | 0.7216 |
| BMI Category | **1.82** | **0.1762*** | 1.7 | 0.1973 |
| Frequency of NSAID Use | 0.11 | 0.9765 | 0.53 | 0.714 |
| Fruit and Vegetable Intake | 0.67 | 0.6148 | **1.94** | **0.1258*** |
| Processed and Red Meat Intake | 0.82 | 0.5209 | 0.28 | 0.8881 |
| Carb Intake | 1.06 | 0.3927 | 0.47 | 0.757 |
| Frequency of Physical Activity | **1.99** | **0.1186*** | 0.78 | 0.5483 |
| Frequency of Stress Reduction | 0.47 | 0.7575 | 0.76 | 0.5587 |
| Alcohol Intake | 0.69 | 0.6066 | 0.58 | 0.6763 |
| Smoking Status | 0.01 | 0.9869 | **6** | **0.0057*** |
| Co-morbidity Count | 0.53 | 0.7813 | **1.76** | **0.1384*** |

Table 3. Association between psychosocial variables with respect to select demographic variables as measured by ANOVA.

*ANOVA significant at P < 0.20

| | Informational Support | | Instrumental Support | | Social Isolation | | Emotional Support | | Companionship | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Demographic Variable** | **F-Value** | **Pr > F** | **F-Value** | **Pr > F** | **F-Value** | **Pr > F** | **F-Value** | **Pr > F** | **F-Value** | **Pr > F** |
| Age Category | 1.59 | 0.2108 | **2.4** | **0.0846*** | **2** | **0.132*** | 0.58 | 0.6349 | **1.92** | **0.1451*** |
| Gender | 0.02 | 0.8791 | 0.86 | 0.3605 | **2.77** | **0.1046*** | **1.76** | **0.1926*** | 0.01 | 0.9237 |
| Ethnicity | **2.17** | **0.1495*** | 1.06 | 0.3103 | 1.07 | 0.3072 | 0.65 | 0.4239 | **1.93** | **0.1729*** |
| Race | 0.33 | 0.7247 | 0.45 | 0.6415 | 0.33 | 0.7222 | 1.54 | 0.2289 | 1.51 | 0.2353 |
| Marital Status | 0.48 | 0.7517 | **3.42** | **0.0188*** | **2.21** | **0.0888*** | 0.08 | 0.9886 | **3.65** | **0.014*** |
| Highest Level of Education | 1.68 | 0.1772 | 0.59 | 0.6727 | 0.55 | 0.7009 | 1.23 | 0.3163 | 1.58 | 0.2012 |
| Employment Status | 1.78 | 0.1442 | 1.24 | 0.3111 | 0.84 | 0.5518 | 0.78 | 0.5938 | 0.73 | 0.6298 |
| Annual Income | 0.52 | 0.8472 | **2.28** | **0.0445*** | **2.13** | **0.0592*** | 1.48 | 0.2008 | **2.17** | **0.0554*** |
| Insurance Coverage | 0.94 | 0.402 | 0.58 | 0.5633 | 0.77 | 0.4695 | 0.67 | 0.5178 | 0.07 | 0.9371 |
| KRAS Mutation | **3.05** | **0.0607*** | **6.87** | **0.003*** | 0.07 | 0.9298 | **2.04** | **0.1453*** | 0.87 | 0.4276 |
| BRAF Mutation | **4.42** | **0.0429*** | **5.15** | **0.0293*** | 0.32 | 0.5724 | 0.59 | 0.449 | **2.1** | **0.156*** |
| Stage (derived) | 0.03 | 0.9943 | 0.33 | 0.8064 | 0.29 | 0.8346 | 0.24 | 0.8666 | 0.17 | 0.9161 |
| Beta-Blocker Use | 0.66 | 0.4216 | 0.04 | 0.843 | 0.18 | 0.6773 | 0.04 | 0.8495 | 0.09 | 0.7716 |
| Anti-Depressant Use | 0.01 | 0.9083 | 0.19 | 0.6678 | 0.02 | 0.8935 | 0.17 | 0.6794 | 0 | 0.9845 |
| Aspirin Use | 1.15 | 0.3421 | 0.6 | 0.6165 | 0.64 | 0.5966 | 0.86 | 0.4718 | 0.62 | 0.6059 |
| Statin Use | 0.14 | 0.7079 | **1.75** | **0.1942*** | **3.28** | **0.0782*** | 0.09 | 0.7628 | 0.77 | 0.3848 |
| BMI Category | 0.11 | 0.8968 | 0.39 | 0.6831 | **2.6** | **0.0885*** | 0.42 | 0.6614 | 0.68 | 0.5115 |
| Frequency of NSAID Use | **1.89** | **0.1348*** | **3.22** | **0.0242*** | 0.32 | 0.8616 | **1.65** | **0.1848*** | **1.64** | **0.1879*** |
| Fruit and Vegetable Intake | 1.17 | 0.3422 | 1.05 | 0.3938 | 1.37 | 0.2649 | 0.89 | 0.4805 | 0.71 | 0.5935 |
| Processed and Red Meat Intake | 0.35 | 0.8417 | **4.52** | **0.0049*** | **3.1** | **0.0282*** | 0.99 | 0.4256 | **2.85** | **0.0387*** |
| Carb Intake | 1.48 | 0.2318 | 1.13 | 0.3578 | 1.41 | 0.2513 | **1.86** | **0.1398*** | 1.13 | 0.3573 |
| Frequency of Physical Activity | 0.26 | 0.8987 | 0.62 | 0.6541 | 0.97 | 0.4342 | 0.76 | 0.5578 | **3.78** | **0.0121*** |
| Frequency of Stress Reduction | 0.59 | 0.6728 | **3.07** | **0.029*** | 0.88 | 0.4881 | 0.69 | 0.6007 | **4.48** | **0.0052*** |
| Alcohol Intake | **2.07** | **0.1079*** | 1.44 | 0.2407 | **2.51** | **0.0598*** | **1.75** | **0.1615*** | 0.84 | 0.5114 |
| Smoking Status | 0.31 | 0.7334 | 0.15 | 0.8578 | **6.25** | **0.0047*** | **2.12** | **0.1343*** | 0.29 | 0.7479 |
| Co-morbidity Count | 0.93 | 0.4877 | 0.88 | 0.5237 | **1.57** | **0.1890*** | 0.68 | 0.6656 | 0.89 | 0.5130 |

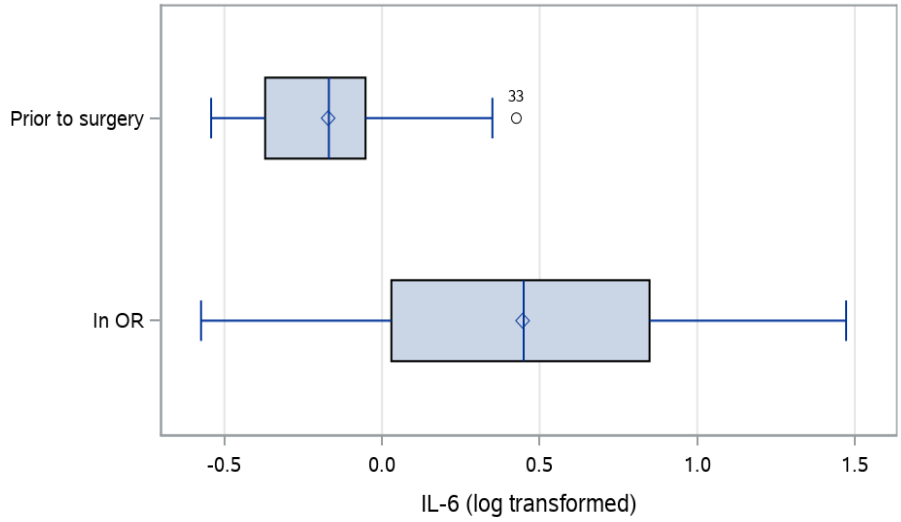
Figure 1. The association between cytokine levels and timing of blood draw. (A) summary table of t-test significance (B) box plot comparing IL-6 log transformed values with respect to draw time (C) box plot comparing IL-8 log transformed values with respect to draw time

*Equality of variance as determined by Folded-F test

(A)

| | Equality of Variances* | p-value |
|---|---|---|
| IL- 6 | Unequal | 0.0867 |
| | | |
| IL-8 | Equal | 0.4783 |

(B)




(C)

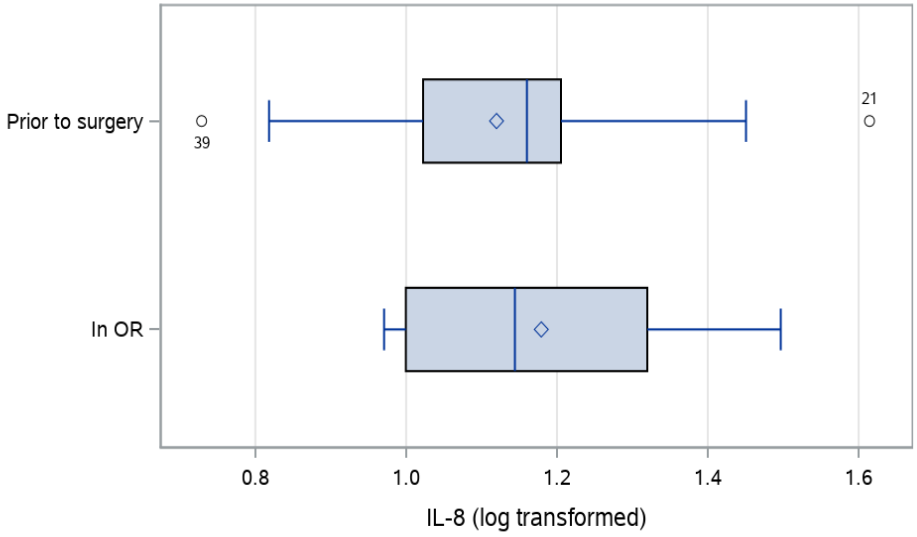


Table 4. Table of Spearman correlation coefficients of psychosocial variables with regards to normalized (A) serum IL-6 and (B) IL-8 values

(A)

| Spearman Correlation Coefficients (P-Value) (n) | | | | | |
|---|---|---|---|---|---|
| | **Informational Support** | **Instrumental Support** | **Social Isolation** | **Emotional Support** | **Companionship** |
| **Serum IL-6 (Log-Transformed)** | -0.30350<br>(0.0640)<br>(38) | 0.03347<br>(0.8397)<br>(39) | 0.02678<br>(0.8715)<br>(39) | -0.19018<br>(0.2462)<br>(39) | -0.24552<br>(0.1319)<br>(39) |

(B)

| Spearman Correlation Coefficients (P-Value) (n) | | | | | |
|---|---|---|---|---|---|
| | **Informational Support** | **Instrumental Support** | **Social Isolation** | **Emotional Support** | **Companionship** |
| **Serum IL-8 (Log-Transformed)** | 0.06363<br>(0.7043)<br>(38) | 0.20231<br>(0.2168)<br>(39) | -0.11369<br>(0.4907)<br>(39) | -0.01842<br>(0.9114)<br>(39) | -0.09031<br>(0.7043)<br>(39) |

Table 5. Simple (univariate) linear regression models

| Independent Variable | Dependent Variable | Parameter Estimate | F-Value | Pr > F |
|---|---|---|---|---|
| Social Isolation | IL-6 | -0.00366 | 0.15 | 0.6993 |
| Companionship | IL-6 | -0.00231 | 0.08 | 0.7732 |
| Instrumental Support | IL-6 | 0.00491 | 0.29 | 0.5929 |
| Informational Support | IL-6 | -0.00951 | 2.19 | 0.1479 |
| Emotional Support | IL-6 | -0.00335 | 0.15 | 0.7016 |
| | | | | |
| Social Isolation | IL-8 | -0.00087 | 0.04 | 0.8394 |
| Companionship | IL-8 | -0.00189 | 0.28 | 0.6031 |
| Instrumental Support | IL-8 | 0.00530 | 1.72 | 0.1981 |
| Informational Support | IL-8 | 0.00152 | 0.18 | 0.6761 |
| Emotional Support | IL-8 | -0.00077 | 0.04 | 0.8467 |

* Parameter estimate for normalized values of bio-markers and socio-emotional variables

Table 6. Multiple linear regression models with relevant covariates included

*Covariates significantly associated with the dependent variable within the model ($p < 0.05$)

| Dependent Variable | Independent Variable | Parameter Estimate | (p-value) | R-Square | F-Value | Pr < F | Covariates Included |
|---|---|---|---|---|---|---|---|
| IL-6 | Informational Support | 0.00873 | 0.1204 | 0.4400 | 4.40 | 0.0044 | Stage, Draw Time* |
| | Instrumental Support | -0.00234 | 0.7924 | 0.5476 | 3.93 | 0.0037 | Stage, Draw Time*, Marital Status |
| | Social Isolation | -0.00262 | 0.8194 | 0.5553 | 3.00 | 0.0134 | Stage, Draw Time*, Marital Status, BMI Category |
| | Emotional Support | 0.01103 | 0.0992 | 0.5506 | 7.11 | 0.0002 | Stage, Draw Time* |
| | Companionship | 0.01128 | 0.1680 | 0.6776 | 3.85 | 0.0030 | Stage, Draw Time*, Marital Status, Frequency of Physical Activity |
| | | | | | | | |
| IL-8 | Informational Support | -0.00295 | 0.4262 | 0.1828 | 1.62 | 0.1954 | Stage |
| | **Instrumental Support** | **-0.01597** | **0.0028** | **0.7905** | **4.24** | **0.0021** | **Stage*, Age Category*, Annual Income*** |
| | Social Isolation | 0.00800 | 0.1356 | 0.6396 | 3.04 | 0.0081 | Age Category, Annual Income, Smoking Status* |
| | Emotional Support | -0.00263 | 0.4642 | 0.4601 | 3.29 | 0.0117 | Stage, Gender*, Smoking Status* |
| | Companionship | -0.00464 | 0.3360 | 0.6691 | 2.28 | 0.0477 | Stage, Age Category, Annual Income* |