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Association Between Inflammatory And Metabolic Biomarkers And Lymphedema In Women With Ovarian Cancer

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Abstract

Background: There is currently no cure for lymphedema, thus being able to predict who is likely to develop lymphedema will allow for early intervention. To date, no study has examined the association between metabolic and inflammatory biomarkers and lower limb lymphedema (LLL) in women with ovarian cancer. This secondary analysis seeks to explore the association between serum blood biomarkers and LLL in women with ovarian cancer and determine if the change in biomarkers over time is associated with a change in lymphedema status.

Methods: Data from the Women's Activity and Lifestyle Study in Connecticut (WALC), a 6-month exercise intervention randomized controlled trial (RCT) in women with ovarian cancer was utilized. LLL assessed via self-report questionnaire, a certified lymphedema therapist, and optoelectronic perometer and blood-based biomarkers (CA-125, CRP, IGF-1, insulin, leptin, adiponectin, IL-6, TNF-α, and VEGF) were reported at baseline and 6-months. Baseline blood biomarkers were reported in their unadjusted form and when adjusting for baseline BMI, chemotherapy, cancer recurrence, and cancer stage using t-tests and ANCOVA, respectively. The change in biomarkers over the 6-month study and the change in lymphedema status was assessed via a generalized linear model.

Results: The sample consisted of 88 women, mean age of 58.1 ± 8.0 years, with 19 women classified as having lymphedema at baseline via the self-report questionnaire. Baseline CA-125 levels were higher in women with lymphedema (21.01 U/mL) compared to those without lymphedema (9.94 U/mL) when adjusting for covariates ($p = 0.043$). A greater increase in levels of CRP ($p = 0.046$) and TNF-α ($p = 0.016$) were associated with the development of lymphedema, according to the self-report questionnaire, which occurred in 8 (11.6%) women during the 6-month study. A greater decrease in levels of CRP ($p = 0.020$), VEGF ($p = 0.031$),

and leptin ($p = 0.022$) were associated with the resolution of lymphedema-like symptoms, according to the self-report questionnaire, which occurred in 10 (52.6%) women.

Conclusion: These results show potential associations between blood biomarker levels and lymphedema status suggesting a mechanistic link as to the etiology of lymphedema. Additional research is needed to further evaluate biomarkers associated with the development of lymphedema in women newly diagnosed with ovarian cancer.

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Introduction

It is estimated that 19,880 women will receive a new diagnosis of ovarian cancer in 2023.[1] As there continues to be advances in cancer treatments, mean survival time of women diagnosed with ovarian cancer has increased.[2] Thus, more women are living with complications of cancer treatment, such as lymphedema.[3]

Computed tomography (CT) scans are utilized to identify tumors based on a women's symptoms, however ovarian cancer is diagnosed through exploratory surgery and tumor debulking.[4] The specific treatment course depends on the stage of cancer and grade of the tumor, with the most common treatment being a hysterectomy with bilateral salpingo-oophorectomy followed by chemotherapy.[4] The approach to completing a hysterectomy varies and can include a total abdominal hysterectomy with either a vertical or a horizontal incision, a vaginal hysterectomy, or a laparoscopic hysterectomy.[5,6] The incisions required for each surgical approach allow for the possibility of damaging different lymph nodes (i.e., paraaortic, retroperitoneal, abdominal, iliac, or inguinal lymph nodes).[7] Any time there is an insult to the lymph nodes, that individual is automatically considered to have Stage 0 lymphedema.[8] The progression of lymphedema is dependent upon the body's ability to get rid of lymphatic fluid.[7] Individuals who have had a regional lymphadenectomy, radiation therapy, or chemotherapy are also at an increased risk of developing lymphedema.[9] To date, there is no cure for lymphedema, only management through complete decongestive therapy.[10] Therefore, finding predictors of lymphedema will allow for early intervention in the management of lymphedema.

Most studies on cancer-related lymphedema have been completed in individuals with breast cancer. Soran et al. conducted a study of predictors of lymphedema in individuals with breast cancer and found that infection, body mass index (BMI), and level of hand use were predictors of

breast-cancer-related lymphedema.[11] Additionally, the results from a case-control study among individuals with breast cancer found BMI to be the only factor associated with lymphedema.[12] Another study in women with breast cancer found that axillary lymph node dissection and radiotherapy were predictive of lymphedema.[13]

When examining lower extremity cancer-related lymphedema, a retrospective cohort study of 413 women with ovarian cancer found the number of lymph nodes resected was significantly associated with lower extremity lymphedema (LLL).[14] Tada et al. completed a retrospective cohort study on 694 women with ovarian and uterine cancer who underwent a pelvic lymph node dissection and found post-operative radiotherapy was significantly associated with lymphedema.[15] In an analysis from the Women's Activity and Lifestyle Study in Connecticut (WALC), Iyer et al. found BMI was the only variable to predict lymphedema in women with ovarian cancer.[16]

Dysfunction of the lymphatic system can lead to altered lipid and protein transport, which results in a progressive inflammatory process.[17, 18, 19, 20] Thus, metabolic and inflammatory biomarkers may be relevant in identifying lymphedema. There have been several studies published on the association of blood biomarkers and lymphedema in individuals with breast cancer as well as head and neck cancer. In women with breast cancer, low pre-surgery levels of IL-1α, IL-6, IL-8, and high pre-surgery levels of VEGF were significantly associated with severe lymphedema at 8-weeks post-surgery.[21] In individuals with head and neck cancer, there was a significant association between IL-6, IL-1β, TNF-α, TGF- β1, and MMP-9 and both overall lymphedema and lymphedema severity.[22] To date, there have not been any studies examining biomarkers and associations with lymphedema in women with ovarian cancer. Research on biomarkers to predict lymphedema in women with ovarian cancer is needed as these women

present with different deficits, such as decreased mobility, compared to individuals with breast or head and neck cancer.

Early intervention is shown to decrease the progression of lymphedema. As there is no current cure for lymphedema, being able to predict who is likely to develop lymphedema will allow for early intervention. Biomarkers may be useful in predicting the development of lymphedema in ovarian cancer patients. Therefore, the aim of this analysis was to determine blood biomarkers associated with inflammation and metabolism that predict the development of lymphedema and severity of lymphedema.

Methods

Study population

The women diagnosed with ovarian cancer included in this analysis (n=88) are a subset of participants from the WALC randomized controlled trial (RCT) (n=144) who completed a clinic visit and had lymphedema and blood biomarker data collected. The WALC study was a 6-month RCT of exercise vs attention control with primary endpoints of health-related quality of life (HRQOL) and cancer-related fatigue (CRF) in women treated for ovarian cancer.[23] In the WALC study, women were randomized in a 1:1 ratio into either a 6-month exercise intervention (n = 74) or an attention-control (n = 70) arm between May 1, 2010 and March 20, 2014. Eligibility for enrollment into this RCT included women ages 18-75 who were English-speaking, diagnosed with ovarian cancer within the previous 4 years, completed chemotherapy at least 1 month prior to randomization, exercised less than 90 minutes per week, and received physician consent for participation. Additional details on the study design, recruitment, and intervention of the WALC study have been previously published.[23]

This secondary analysis was limited to women enrolled in WALC that completed a baseline and 6-month clinic visit at Yale-New Haven Hospital (n=95) because the assessment of LLL was limited to this subgroup. Out of these 95 women, only 88 had biomarker data available (Figure 1). These women were recruited using the Rapid Case Ascertainment Shared Resource of the Yale Cancer Center—a Connecticut Tumor Registry resource that identified women from all hospitals in Connecticut. All study procedures were approved by the human investigations committee at Yale University and the Connecticut Department of Public Health, and the institutional review boards at 21 Connecticut hospitals.

Baseline measurements

Randomization to the intervention or attention-control group was lost since data were analyzed to determine lymphedema status among all participants regardless of intervention arm. Baseline characteristics, of all 88 women with lymphedema and biomarker data available, were assessed at study enrollment (Table 1). Sociodemographic information was collected via self-report at baseline. Diagnosis and treatment information were obtained via self-report and then verified by the participants' oncologist. Height and weight were measured with women wearing light clothing without shoes, and measurements were rounded to the nearest 0.5 cm and 0.01 kg, respectively. The Modifiable Physical Activity Questionnaire, which gathers information on the duration and frequency of 20 recreational activities during the previous 6 months, was used to assess baseline physical activity levels.[24] Total fat mass was measured by whole-body DEXA scans (Hologic QDR 1500, Hologic Inc., Waltham, Mass) with participants following standard guidelines for food and drug intake. All DEXA scans were evaluated by a radiologist blinded to the study arm.

Lower Limb Lymphedema Assessments

The WALC study found substantial agreement between a certified lymphedema therapist measuring lymphedema – the current gold standard – and measuring lymphedema through self-report.[16] There was no agreement between measuring lymphedema through optoelectronic perometry and a certified lymphedema therapist.[16] Due to these findings, this analysis uses lymphedema status as classified by self-report to assess change in lymphedema status over time as the majority of women completed this assessment.

Self-report questionnaire

The Norman Lymphedema Questionnaire is an interview-administered questionnaire that was originally developed to examine the incidence and degree of lymphedema in women with breast cancer.[25] This questionnaire was adapted for the WALC study to examine LLL in women with ovarian cancer. This interview-administered questionnaire asked a series of questions about self-observed differences in their feet, lower legs, upper legs, and abdomen after treatment for ovarian cancer. In this study, the presence of LLL was defined as any self-reported difference in size, heaviness, swelling, and induration between lower limbs that was not present before treatment for ovarian cancer.

Responses for the question, "between the time of ovarian cancer diagnosis (the reference date) and the interview date did their right and left legs differ in size". This question was asked separately for the foot, lower leg, upper leg, and abdomen. Women rated differences as "1: very slight; you are the only person who would notice this"; "2: noticeable to people who know you well but not to strangers"; or "3: very noticeable." Based on these responses, the degree of swelling was then summed with a potential range of 0-12. A score of more than 0 indicated any lymphedema, a score of 1-4 indicated mild lymphedema, and a score of 5-12 indicated

moderate/severe lymphedema. For a score of 5 or more, individuals also needed a measured size difference at two or more locations (i.e., feet, lower legs, upper legs, or abdomen).

Certified lymphedema therapist

A lymphedema therapist, certified by the Lymphology Association of North America, assessed the presence of lymphedema through a series of questions and a physical examination. The series of questions included 1) whether they had a history of swelling in their legs and noted any perceived changes since surgery, 2) whether swelling occurred with physical activity, and 3) whether there were changes in the appearance of their legs throughout the day. The lymphedema therapist then performed a physical examination and assessed the degree of pitting or induration in both legs and visually noted differences between the right and left leg. A woman was classified as either having lymphedema or not having lymphedema by the lymphedema therapist after assessment. If a woman had pitting edema, palpable induration, a history of swelling both with and without completion of physical activity, self-reported changes in appearance between lower limbs before and after treatment for ovarian cancer, and visual differences between limbs, the woman was classified as having lymphedema.

Optoelectronic perometer

An optoelectronic perometer is a device utilized to measure limb volume. This technique for measuring limb volume has been extensively studied for both validity and reliability. The optoelectronic perometer has high intra-rater reliability (interclass correlation (ICC): 0.989; 95% confidence interval (CI): [0.99, 0.99]) and high inter-rater reliability (ICC: 0.993; 95% CI: [0.99, 1.01]) when compared to other volumetric assessments for lymphedema.[26, 27] When standing, the certified lymphedema therapist had the women place one leg at a time in the optoelectronic perometer and the circumferential measurements of each leg was taken every 4 cm.[26, 27] Based on

these measurements, the software generated the volume of both lower limbs.[26, 27] According to limb volume measurements from the optoelectronic perometer, the presence of lymphedema was defined as an inter-limb volume discrepancy of 5% or more. This definition is consistent with the standard definitions outlined by the International Society of Lymphology.[28]

The self-report questionnaire, assessment by a certified lymphedema therapist, and measurements from the optoelectronic perometer were conducted at baseline and again at 6-months. The same lymphedema therapist performed the lymphedema assessment at both time points. The sub-study assessing LLL via a certified lymphedema therapist and optoelectronic perometer began 1 year after the commencement of the WALC study, therefore only 56 women (64%) received these evaluations at baseline.

Biomarkers

At baseline and 6-months, a 12-hour fasting blood draw was performed. The blood was processed, and serum was stored at -80°C until assayed. Serum concentrations of insulin, leptin, and adiponectin were measured using radioimmunoassay kits (RIA kits from EMD Millipore); cancer antigen-125 (CA-125), insulin-like growth factor-1 (IGF-1), interleukin-6 (IL-6), and tumor necrosis factor alpha (TNF-α) were measured using ELISA kits (R&D Systems, Minneapolis, MN); vascular endothelial growth factor (VEGF) was measured using ELISA kit (Thermofisher Scientific Inc, Waltham, MA); and C-reactive protein (CRP) was measured using an automated chemistry analyzer. Biomarker samples collected at baseline and at 6 months were analyzed simultaneously at the end of the study by laboratory technicians who were blinded to treatment assignment. The samples were measured in duplicate, and the coefficients of variation (CV) were all under 10%. Additionally, the intra-assay CV for all assays were less than 5%.

Statistical analyses

Baseline descriptive statistics were reported for the total sample. A Student's t-test, Chi-square test, or Fisher's exact test were used to compare baseline demographics and clinical characteristics among women with lymphedema and those without lymphedema as assessed via the self-report questionnaire. Iyer at al. found that there was substantial agreement ($\kappa = 0.61$) between the self-report questionnaire and assessment by the lymphedema therapist—which is the current gold standard measure to assess lymphedema.[16] Due to these findings, the classification of lymphedema as assessed via the self-report questionnaire was utilized to report baseline characteristics between groups since all 88 women completed this measure.

The presence of lymphedema as assessed via each of the 3 measurements were descriptively reported, and the number of women that were classified as having lymphedema according to any of the 3 measurements were reported. Lymphedema severity was calculated using responses from the adapted Norman Lymphedema Questionnaire and women with lymphedema were classified as either having mild or moderate/severe lymphedema. The change in lymphedema status from baseline to 6-months was calculated according to lymphedema status as assessed via the self-report questionnaire and was descriptively reported.

Analysis of CA-125, CRP, insulin, IGF-1, and leptin were prespecified, however analysis of adiponectin, IL-6, TNF-α, and VEGF were unplanned and thus considered exploratory analyses. The baseline levels of biomarkers were first reported without adjusting for potential confounders utilizing a Student’s t-test. Baseline biomarker levels were then reported when adjusting for baseline BMI, chemotherapy, cancer recurrence, and cancer stage using an analysis of covariance (ANCOVA) model. BMI was found to be a significant predictor of LLL, therefore it was controlled for when reporting baseline biomarkers levels.[16] Multiple studies have shown

that chemotherapy, cancer recurrence, and cancer stage influence the presence of lymphedema; therefore, these factors were also controlled for in the analyses.[11, 12, 13, 14, 15] The mean change in biomarker levels from baseline to 6-months was calculated. A generalized linear model was fit to determine whether a change in lymphedema status was associated with the 6-month change in biomarker levels.

Effect size was reported using Hedge's g for Student's t-test and odds ratio (OR) for Chi-square and Fisher's exact test. All analyses were completed using SAS Version 9.4 (SAS, Cary, North Carolina).[29] Tests were 2-sided, and the threshold for statistical significance was < 0.05.

Results

Among the 144 women enrolled in the WALC study, 95 women completed a clinic visit at Yale-New Haven Hospital, and 88 women had lymphedema and biomarker data available and thus were included in this analysis (Figure 1). The women had a mean age of 58.1 ± 8.0 years and the sample consisted primarily of non-Hispanic white (n = 84; 95.5%) women. The majority of the women had a college degree (n = 44; 50%), were unemployed or retired (n = 40, 45.5%), and were married or living with their partner (n = 65; 73.9%). At baseline, the average BMI was 29.3 ± 7.0 kg/m^2 and the average percent body fat was 40.1% ± 5.6%. On average, at baseline the women reported 26 ± 37.9 minutes of physical activity per week. Most of the women had no family history of ovarian cancer (n = 72; 81.8%), did not have cancer recurrence prior to study enrollment (n = 67; 76.1%), and had been diagnosed with ovarian cancer for an average of 1.6 ± 0.9 years prior to study enrollment. Twenty-one of the women (23.9%) were diagnosed with stage I cancer, 21 (23.9%) with stage II, 30 (34.1%) with stage III, and 16 (18.2%) with stage IV ovarian cancer. Some women reported having more than one surgical procedure. However, the most common procedures were total abdominal hysterectomy (n = 89; 94.3%), bilateral salpingo-

oophorectomy (n = 81; 92.1%), omentectomy (n = 76; 83%), and a lymph node dissection (n = 73; 83.0%). Demographics and clinical characteristics between women with and without lymphedema at baseline were similar. The only characteristic that differed between groups was cancer stage (p = 0.007). More women with lymphedema at baseline had stage I ovarian cancer (52.6% vs 15.9%), whereas fewer women with stage II (5.3% vs 29.0%) and stage III ovarian cancer (26.3% vs 36.2%) had lymphedema. Additional demographic and clinical characteristics of the sample are listed in Table 1.

Lower limb lymphedema prevalence

At baseline, all 88 women completed the self-report questionnaire, and according to this measure 21.6% (n = 19) were classified as having lymphedema (Table 2). Women could report lymphedema-like symptoms in more than one region of their lower limbs and abdomen. Among the 21.6% of women that were classified as having lymphedema at baseline, 10.2% (n = 9) women reported lymphedema-like symptoms in their feet, 12.5% (n = 11) women reported lymphedema-like symptoms in their lower legs, 2.3% (n = 2) women reported lymphedema-like symptoms in their upper legs, and 10.2% (n = 9) women reported lymphedema-like symptoms in their abdomen. Of the 56 women with clinic assessments completed, the lymphedema therapist classified 11.4% (n = 10) as having lymphedema and 13.6% (n = 12) were classified as having lymphedema based on the optoelectronic perometer assessment (Table 2). The women classified as having lymphedema using the optoelectronic perometer had an average inter-limb volume difference of 6.1% (SD 1.0; range 5.0% – 7.7%), thus were all classified as having mild lymphedema—an inter-limb difference between 5% and 9%.[28] Overall, 36.4% (n = 32) of women were classified as having lymphedema as assessed by at least 1 of the 3 measurements.

According to the self-reported questionnaire, 3% (n = 3) had moderate/severe lymphedema, and 18% (n = 16) had mild lymphedema at baseline (Table 3).

At the 6-month follow-up, the prevalence of LLL decreased according to the self-report questionnaire and lymphedema specialist and stayed the same based on optoelectronic perometer assessment (Table 2). As referenced in Table 2, 6 fewer women were classified as having lymphedema via self-report and 2 fewer women were classified as having lymphedema by a lymphedema therapist at 6 months. Twenty-five percent (n = 22) of the women were classified as having lymphedema as assessed by at least 1 of the 3 measurements at 6 months, compared to 36.4% (n = 32) at baseline.

Baseline biomarker levels

At baseline, there were no statistically significant differences in blood biomarker levels between those with and without lymphedema assessed by self-report, lymphedema specialist, or optoelectronic perometry. With a priori adjustment for potential confounders (baseline BMI, chemotherapy, cancer recurrence, and cancer stage), CA-125 was the only biomarker that was statistically significantly different between those with lymphedema (mean ± SD = 21.05 ± 45.69 U/mL) and those without lymphedema (mean ± SD = 9.94 ± 16.47 U/mL) (F-value = 4.26; p = 0.043; Table 4), as classified according to the self-report questionnaire. With lymphedema status classified via lymphedema therapist or optoelectronic perometer, there were no statistically significant differences in baseline biomarker levels when adjusting for potential confounders (Table 5 and 6).

6-month change in lymphedema status and biomarker levels

Twenty-four percent (18 out of 75) of women had a change in their lymphedema status from baseline to 6-months (Table 7). A total of 13 women did not fill out the 6-month self-report

questionnaire and thus are classified as missing. Among women who developed lymphedema over the 6-month study (n = 8) based on the self-report questionnaire, only the change in CRP (F-value = 4.14; p = 0.046) and TNF-α (F-value = 6.06; p = 0.016) levels from baseline to 6-months were associated with the change in lymphedema status. From baseline to 6-months, the mean increase in CRP was 6.29 mg/L (SD 21.81) and the mean increase in TNF-α was 0.29 pg/mL (SD 0.48) among those who developed lymphedema over the 6-month study compared to a mean increased in CRP of 0.33 mg/L (SD 5.18) and a mean decrease in TNF-α of 0.02 pg/mL (SD0.31) in those who did not develop lymphedema during the 6-month study. Among women who had lymphedema at baseline and then were classified as not having lymphedema at 6-months (n = 10) according to the self-report questionnaire, the change in CRP (F-value = 5.65; p = 0.020), leptin (F-value = 5.48; p = 0.022), and VEGF (F-value = 4.84; p = 0.031) levels from baseline to 6-months were associated with the change in lymphedema status. From baseline to 6-months, CRP levels decreased by an average of 6.18 mg/L (SD -29.58), leptin levels decreased by an average of 14.11 ng/mL (SD 24.67), and VEGF levels decreased by an average of 74.44 pg/mL (SD 129.32) among those who had lymphedema initially and then were found to not have lymphedema at 6 months compared to a mean increase in CRP levels by 0.53 mg/L (SD 1.31), decrease in leptin levels by 6.71 ng/mL (SD 10.19), and increase in VEGF levels by 43.85 pg/mL (SD 148.79) among those who had lymphedema both at baseline and 6 months (Table 8).

Discussion

Lymphedema is an incurable side effect of ovarian cancer treatment; therefore, it is critical to identify factors that are associated with the development of lymphedema to provide targeted education and early intervention. The WALC study was the first study to assess the

prevalence of LLL in women post-treatment for ovarian cancer.[16] To our knowledge, there are no studies looking at biomarkers associated with LLL in women with ovarian cancer.

In our study, women with lymphedema had significantly higher baseline levels of CA-125 compared to those without lymphedema (as classified by the self-report questionnaire). Literature suggests that CA-125 is the most promising biomarker for screening, detecting, and managing ovarian cancer, with elevated CA-125 levels being associated with ovarian cancer and ovarian cancer recurrence.[30] Change in CRP levels over the 6-month study was associated with the bidirectional change in lymphedema status. Increased CRP levels in the blood are indicative of inflammation in the body.[31] Therefore, this finding may suggest that higher CRP levels are associated with the presence of lymphedema. Previous studies in women with breast cancer suggest that higher CRP levels are associated with the presence of lymphedema and with worse prognosis, however more research is needed to confirm causality.[32]

Among women who developed lymphedema during the 6-month study, an increase in TNF-α levels were also shown to be associated with change in lymphedema status; although, we did not see a statistically significant decrease in TNF-α among women changing from having lymphedema to not having lymphedema. TNF-α is an inflammatory cytokine that increases during periods of acute inflammation.[33] This potentially explains why we saw an increase in TNF-α levels among women who developed lymphedema over the 6-month time period and not among those whose presence of lymphedema-like symptoms resolved. Additionally, among women who developed lymphedema during the 6-month study, a decrease in leptin levels were shown to be associated with change in lymphedema status; however, we did not see a statistically significant increase in leptin among women changing from not having lymphedema to having lymphedema. Leptin is a hormone that is produced by adipose tissue and has been shown to be

higher in individuals who are overweight or obese and among individuals who develop lymphedema postoperatively.[34] There are two potential explanations for the significant decrease in leptin levels among women who had a resolution of lymphedema-like symptoms over the course of this 6-month study. First, this RCT was an exercise intervention study; therefore, it is possible that women enrolled in the intervention arm of this trial experienced decreased leptin levels and the diminished presence of detectable LLL as a result of participation in more exercise. However, among women who had a resolution of lymphedema-like symptoms, leptin levels decreased over the 6-month study in both those enrolled in the exercise intervention (n=5) and to the attention-control (n=5); however, the significance of these findings are limited because of the very small sample size. Second, women enrolled in the study were post treatment for ovarian cancer, thus as time passed since their cancer treatment surgery leptin levels decreased. Additional research needs to be completed to test both of these potential hypotheses at diagnosis and before and after surgery and chemotherapy.

A previous study in women with breast cancer found that pre-surgery IL-6 and VEGF levels were associated with the development of breast cancer-related lymphedema.[21] Although this analysis did not obtain pre-treatment biomarker levels, we did find that the resolution of lymphedema-like symptoms was associated with a decrease in VEGF levels ($p = 0.031$). And although not statistically significant, the development of lymphedema over the 6-month study was associated with an increase in VEGF levels ($p = 0.061$). These sample sizes were very small, therefore with a larger sample size we may be more likely to detect a significant association. A mouse tail lymphedema model study found that increased VEGF levels led to aggravation of lymphedema.[35] Conversely, decreased VEGF levels were associated with a reduction in edema.[35]

Although these results were from a mouse model, the increase and decrease in VEGF levels are similar to our findings in women with ovarian cancer.

In a study in individuals with head and neck cancer, they found IL-6 and TNF-α to be significantly associated with both the development of lymphedema and lymphedema severity.[22] One finding from this study was consistent with our findings. Increased TNF-α levels from baseline to 6-months were associated with the development of lymphedema ($p = 0.016$). We were unable to determine which biomarkers were associated with increased lymphedema severity due to the very small sample of women with lymphedema.

A previous WALC analysis found that a 6-month exercise intervention led to decreased prevalence of LLL.[16] And an additional analysis showed that post intervention IGF-1 (least squared means (95% confidence interval), -14.2 (-26.1, -2.3)) and leptin (-8.9 (-16.5, -1.4)) were significantly reduced in the exercise group compared to those in attention-control.[36] Due to the small number of women with lymphedema in the exercise and attention-control arms of this study, we were unable to perform additional analyses to assess the association between the change in biomarkers and the change in lymphedema status when stratified by exercise intervention. A future study with a much larger sample of women with lymphedema would be needed to determine if an association was present.

Limitations of this study need to be considered when interpreting the results. The small sample size may have limited our ability to detect an association between biomarker levels and the presence of lymphedema. The WALC study enrolled 144 women, which is a relatively large sample size for this type of cancer and intervention, however lymphedema assessments and biomarker levels were only collected from the women enrolled in the study at Yale-New Haven Hospital, which significantly reduced the sample size. Many studies on breast cancer-related

lymphedema reported radiation as a predictor of lymphedema, however information on duration and frequency of radiation therapy was not obtained for this study; thus, we were unable to control for radiation status in the analysis. Future recruitment efforts should focus on enrolling women of various races and ethnicities to determine if these findings differ by race and ethnicity.

There are no other studies in women with ovarian cancer to compare our findings to, therefore a much larger scale study is needed to further identify biomarkers that are associated with lymphedema. With a larger scale study in women with ovarian cancer-related lymphedema, we may be able to determine which serum biomarkers are associated with increased lymphedema severity. More severe lymphedema is shown to be associated with poorer quality of life and decreased ability to complete activities of daily living and instrumental activities of daily living.[37] Future studies should attempt to measure factors associated with increased severity of ovarian cancer-related lymphedema in order to provide education and early invention with a goal of limiting the functional impact lymphedema has on women with ovarian cancer.

Conclusion

In summary, we found that CRP and TNF-α were associated with the development of lymphedema, and CRP, VEGF, and leptin were associated with the resolution of lymphedema-like symptoms. Future studies should focus on examining serum biomarkers at diagnosis and their association with lymphedema after surgery and treatment. Being able to further identify serum blood biomarkers that are associated with the development of lymphedema in women with ovarian cancer will allow for early education and intervention.

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Appendix

Figure 1. Consort diagram

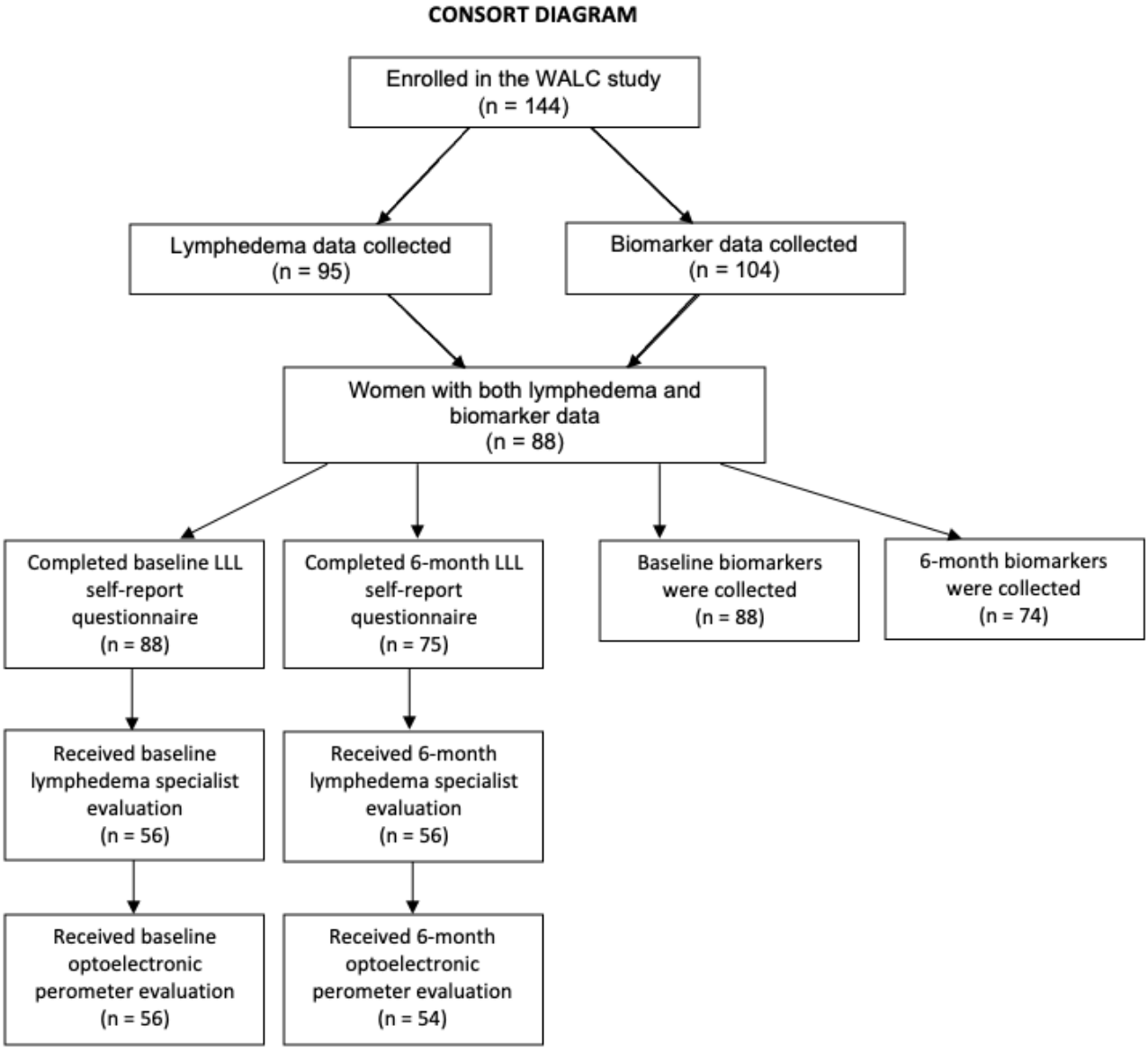

Table 1: Descriptive Statistics

Characteristic	Total Sample (N = 88) [a]	Lymphedema[b] (N = 19) [a]	No Lymphedema[b] (N = 69) [a]	P-value[c]
Age (years)	58.1 (8.0)	57.3 (8.0	58.3 (8.0)	0.640
Range	41 - 75	45 - 71	41 -75	
Race				
White	87 (98.9%)	19 (100.0%)	68 (98.6%)	0.784
Ethnicity				
Non-Hispanic	84 (95.5%)	19 (100.0%)	65 (94.2%)	0.371
Hispanic	4 (4.6%)	0 (0%)	4 (5.8%)	
Education Level				
≤ High school education	15 (17.1%)	2 (10.5%)	13 (18.8%)	0.534
Some college	29 (33.0%)	8 (42.1%)	21 (30.4%)	
College degree	44 (50.0%)	9 (47.4%)	35 (50.7%)	
Employment Status				
Unemployed/Retired	40 (45.5%)	7 (38.9%)	33 (47.8%)	0.207
Employed Part Time (<35 hrs/wk)	18 (20.5%)	2 (11.1%)	16 (23.2%)	
Employed Full Time (>35 hrs/wk)	29 (33.0%)	9 (50%)	20 (29.0%)	
Marital Status				
Single, Divorced/Separated, or Widowed	23 (26.1%)	7 (36.8%)	16 (23.2%)	0.111
Married or Living with Partner	65 (73.9%)	12 (63.2%)	53 (76.8%)	
Family History of Ovarian Cancer				
Yes	14 (15.9%)	3 (16.7%)	11 (16.2%)	0.960
No	72 (81.8%)	15 (83.3%)	57 (83.8%)	
Stage				
I	21 (23.9%)	10 (52.6%)	11 (15.9%)	0.007
II	21 (23.9%)	1 (5.3%)	20 (29.0%)	
III	30 (34.1%)	5 (26.3%)	25 (36.2%)	
IV	16 (18.2%)	3 (15.8%)	13 (18.8%)	
Chemotherapy Prior to Enrollment				
Yes	81 (92.1%)	16 (84.2%)	65 (94.2%)	0.132
No	7 (8.0%)	3 (15.8%)	4 (5.8%)	
Surgical Procedure[d]				
Total Abdominal Hysterectomy	83 (94.3%)	16 (84.2%)	67 (97.1%)	0.058
Bilateral Salpingo-Oophorectomy	81 (92.1%)	17 (94.4%)	64 (95.5%)	0.426
Omentectomy	76 (86.4%	17 (100.0%)	59 (90.8%)	0.236
Lymph Node Dissection	73 (83.0%)	18 (94.7%)	55 (83.3%)	0.155
Tumor Debulking	31 (35.2%)	6 (33.3%)	25 (41.0%)	0.559
Small Bowel Resection	7 (8.0%)	1 (6.3%)	6 (10.0%)	0.366
Colon Resection	9 (10.2%)	2 (11.8%)	7 (11.7%)	0.326
Cancer Recurrence Prior to Enrollment				
Yes	9 (10.2%)	2 (12.5%)	7 (11.7%)	0.325
No	67 (76.1%)	14 (87.5%)	53 (88.3%)	
Time since diagnosis (years)	1.6 (0.9)	1.5 (0.9)	1.6 (0.9)	0.596
Body Mass Index (wt/ht^2(kg/m^2))	29.3 (7.0)	28.8 (7.0)	29.5 (7.0)	0.721
Percent Body Fat (DEXA)	40.1 (5.6)	39.1 (5.2)	40.3 (5.7)	0.381
Physical Activity (min/wk)	26.0 (37.9)	31.6 (41.6)	24.5 (37.0)	0.471
Smoking Status				
Never	45 (51.1%)	9 (47.4%)	36 (52.2%)	0.183
Former	19 (21.6%)	2 (10.5%)	17 (24.6%)	
Current	24 (27.3%)	8 (42.1%)	16 (23.2%)	

[a]: Numbers may not sum to total due to missing data, and percentages may not sum to 100% due to rounding; Table values are mean ± SD or n (%)

[b]: Lymphedema status assessed via self-reported questionnaire

[c]: P-value is for t-test and chi-squared test or Fisher's exact test

[d]: The surgical procedures are not mutually exclusive

Table 2. Prevalence of Lymphedema at Baseline and 6 Months by Assessment Measure

Baseline N (%)[a]			6-months N (%)[a]		
Self-Report Questionnaire (n=88)	**Optoelectronic Perometer** (n=56)	**Lymphedema Therapist** (n=56)	**Self-Report Questionnaire** (n=75)	**Optoelectronic Perometer** (n=54)	**Lymphedema Therapist** (n=56)
19 (21.6%)	12 (13.6%)	10 (11.4%)	13 (14.8%)	12 (13.6%)	8 (9.1%)
Any Diagnosis of Lymphedema (n=88)			**Any Diagnosis of Lymphedema** (n=88)		
32 (36.4%)			22 (25.0%)		

Table 3. Lymphedema severity assessed via The Norman Lymphedema Questionnaire

	Baseline[a] (n=88)	**6-months**[a] (n = 69)
Lymphedema Severity		
None	66 (78%)	60 (87%)
Mild	16 (18%)	8 (12%)
Moderate/Severe	3 (3%)	1 (1%)

[a] Table values are n (%)

Table 4. Adjusted baseline biomarker levels by lymphedema status, assessed via self-reported questionnaire

Biomarker	Lymphedema[a] (n=19)	No Lymphedema[a] (n=69)	P-value[b]
Prespecified			
CA-125 (U/mL)			
Baseline	21.05 (45.69)	9.94 (16.47)	0.043
CRP (mg/L)			
Baseline	3.66 (4.08)	4.75 (5.73)	0.470
IGF-1 (ng/mL)			
Baseline	78.98 (29.91)	84.57 (38.97)	0.675
Insulin (μU/mL)			
Baseline	13.13 (5.86)	15.34 (11.30)	0.197
Leptin (ng/mL)			
Baseline	35.13 (22.82)	33.75 (21.83)	0.897
Exploratory			
Adiponectin (μg/mL)			
Baseline	16.33 (12.88)	18.82 (9.67)	0.623
IL-6 (pg/ml)			
Baseline	2.03 (1.23)	2.02 (1.28)	0.930
TNF-α (pg/mL)			
Baseline	1.19 (0.50)	1.17 (0.37)	0.987
VEGF (pg/mL)			
Baseline	303.18 (268.91)	258.33 (200.76)	0.565

[a] Table values are mean ± SD when adjusting for baseline BMI, chemotherapy, cancer recurrence, and cancer stage
[b] P-value is for ANCOVA

Table 5. Adjusted baseline biomarker levels by lymphedema status, assessed via lymphedema therapist

Biomarker	Lymphedema[a] (n=10)	No Lymphedema[a] (n=46)	P-value[b]
Prespecified			
CA-125 (U/mL)			
Baseline	7.89 (8.05)	11.17 (18.66)	0.863
CRP (mg/L)			
Baseline	5.49 (5.62)	4.29 (5.79)	0.621
IGF-1 (ng/mL)			
Baseline	76.56 (29.72)	83.34 (38.13)	0.740
Insulin (μU/mL)			
Baseline	15.06 (7.16)	15.52 (11.57)	0.491
Leptin (ng/mL)			
Baseline	35.87 (21.55)	35.46 (24.19)	0.503
Exploratory			
Adiponectin (μg/mL)			
Baseline	17.92 (15.34)	17.65 (10.13)	0.582
IL-6 (pg/ml)			
Baseline	2.07 (1.21)	2.00 (1.32)	0.845
TNF-α (pg/mL)			
Baseline	1.03 (0.29)	1.13 (0.40)	0.279
VEGF (pg/mL)			
Baseline	262.38 (207.17)	246.98 (177.84)	0.853

[a] Table values are mean ± SD when adjusting for baseline BMI, chemotherapy, cancer recurrence, and cancer stage
[b] P-value is for ANCOVA

Table 6. Adjusted baseline biomarker levels by lymphedema status, assessed via optoelectronic perometer

Biomarker	Lymphedema[a] (n=12)	No Lymphedema[a] (n=44)	P-value[b]
Prespecified			
CA-125 (U/mL)			
Baseline	9.61 (11.36)	10.84 (18.61)	0.870
CRP (mg/L)			
Baseline	6.82 (5.95)	3.93 (5.59)	0.356
IGF-1 (ng/mL)			
Baseline	76.91 (28.55)	83.40 (38.51)	0.917
Insulin (μU/mL)			
Baseline	20.10 (18.08)	14.16 (7.72)	0.477
Leptin (ng/mL)			
Baseline	42.72 (22.54)	33.53 (23.67)	0.631
Exploratory			
Adiponectin (μg/mL)			
Baseline	12.50 (7.18)	19.11 (11.57)	0.306
IL-6 (pg/ml)			
Baseline	2.03 (0.52)	2.01 (1.42)	0.573
TNF-α (pg/mL)			
Baseline	1.30 (0.56)	1.06 (0.30)	0.111
VEGF (pg/mL)			
Baseline	315.38 (218.23)	231.47 (168.32)	0.338

[a] Table values are mean ± SD when adjusting for baseline BMI, chemotherapy, cancer recurrence, and cancer stage
[b] P-value is for ANCOVA

Table 7. Change in lymphedema status from baseline to 6-months, assessed via self-report questionnaire

Lymphedema Status	n (%)
Change in lymphedema status from baseline to 6-months	18 (24%)
Lymphedema at baseline → No lymphedema at 6-months	10 (11%)
No lymphedema at baseline → Lymphedema at 6-months	8 (9%)
No change in lymphedema status from baseline to 6-months	57 (76%)
Lymphedema at both baseline and 6-months	5 (6%)
No lymphedema at baseline or at 6-months	52 (59%)
Missing	
Lymphedema at baseline → Missing data at 6-months	4 (5%)
No lymphedema at baseline → Missing data at 6-months	9 (10%)

Table 8. Change in lymphedema status associated with change in biomarker level

Six-month change in biomarker levels	Change from not having lymphedema at baseline to having lymphedema at 6-months (n = 8)			Change from having lymphedema at baseline to not having lymphedema at 6-months (n = 10)			No change in lymphedema status: lymphedema at baseline and 6-months (n=5)			No change in lymphedema status: no lymphedema at baseline or 6-months (n=52)		
	Mean Change (SD)	**F-value**	**P-value**	**Mean Change (SD)**	**F-value**	**P-value**	**Mean Change (SD)**	**F-value**	**P-value**	**Mean Change (SD)**	**F-value**	**P-value**
CA-125	-1.98 (3.55)	0.27	0.603	2.46 (9.60)	1.36	0.247	6.13 (10.09)	1.24	0.268	-10.38 (31.16)	3.14	0.081
CRP	6.29 (21.81)	4.14	0.046	-6.18 (-29.58)	5.65	0.020	0.53 (1.31)	0.01	0.917	0.33 (5.18)	0.09	0.767
IGF-1	3.49 (32.82)	0.05	0.821	-4.52 (23.24)	0.34	0.564	-5.38 (28.60)	0.21	0.650	0.82 (33.28)	0.01	0.940
Insulin	-4.76 (9.60)	2.00	0.162	-1.20 (5.01)	0.02	0.881	4.27 (7.31)	3.88	0.053	-1.52 (6.59)	0.00	0.986
Leptin	-3.61 (15.37)	0.24	0.626	-14.11 (24.67)	5.48	0.022	-6.71 (10.19)	0.52	0.472	3.65 (20.71)	5.79	0.019
Adiponectin	-2.35 (5.08)	1.56	0.216	-0.43 (4.93)	0.32	0.575	-5.89 (8.37)	4.28	0.042	2.33 (8.10)	6.19	0.015
IL-6	0.12 (1.01)	0.08	0.774	-0.57 (1.12)	2.06	0.156	-0.85 (1.82)	1.90	0.173	0.14 (1.28)	2.11	0.151
TNF-α	0.29 (0.48)	6.06	0.016	-0.06 (0.41)	0.50	0.484	0.01 (0.39)	0.00	0.980	-0.02 (0.31)	1.45	0.232
VEGF	49.41 (91.32)	3.61	0.061	-74.44 (129.32)	4.84	0.031	43.84 (148.79)	1.74	0.191	-16.94 (80.88)	0.34	0.562