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The pro-inflammatory effect of obesity on high grade serous ovarian cancer

Camille C. Gunderson [a,*], Kai Ding [b], Justin Dvorak [b], Kathleen N. Moore [a], D. Scott McMeekin [a], Doris M. Benbrook [a]

[a] Section of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Oklahoma, 800 NE 10th Street, Oklahoma City, OK, USA
[b] Department of Biostatistics and Epidemiology, University of Oklahoma, 800 NE 10th Street, Oklahoma City, OK, USA

HIGHLIGHTS

- Positive correlations between serum cytokines diminished with obesity.
- Serum cytokines were not associated with platinum sensitivity.
- VEGF correlated with overall survival in high grade serous ovarian cancer.

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ABSTRACT

Introduction. Obesity is a known generator of chronic inflammation but has an uncertain role in ovarian carcinogenesis and survival. Pro-inflammatory cytokines have previously been associated with poor outcomes. Given the established links, we sought to determine whether obesity and pro-inflammatory cytokines affect platinum sensitivity.

Methods. A retrospective review was performed of patients undergoing primary debulking surgery (PDS) for high grade serous ovarian cancer (HGSC) who had available pre-operative serum. Oncologic and treatment characteristics were recorded and analyzed using SAS version 9.3. Bioplex reagent kit was used to measure serum cytokine concentrations.

Results. 86 patients met study criteria. Most were Caucasian (88%) and non-diabetic (92%). All patients had advanced stage (III/IV) disease and received chemotherapy after PDS. In univariate analysis, lower VEGF ($p = 0.013$) was associated with longer overall survival (OS). Low IL-8 level ($p = 0.053$) was marginally associated with platinum resistant disease. After adjusting for covariates including residual disease and maintenance therapy, IL-8 was no longer associated with platinum sensitive status ($p = 0.13$), VEGF remained associated with OS (low vs. high HR 0.3, 95% CI 0.1–0.8, $p = 0.018$), and higher IL-12 was associated with longer PFS (HR 0.4, 95% CI 0.2–0.9, $p = 0.031$).

Conclusion. In HGSC, pro-inflammatory cytokines are influenced by obesity, as differing inter-cytokine correlations were observed based on BMI, possibly due to dysregulation between cytokines in the setting of obesity. Differences in survival and platinum sensitivity were not noted. Future studies are warranted to determine whether obesity may be a modifiable risk factor for poorer outcomes due to differing immune response.

1. Introduction

Obesity has grown to become a massive health epidemic in the United States with a dramatic increase in prevalence over the past 20 years. Greater than one-third (35%) of adults are obese (defined as body mass index [BMI] ≥ 30 kg/m^2), including 36.5% of women [1]. Obesity is known to increase all-cause mortality due to death from cardiovascular disease [2]. However, the link between obesity and a greater incidence of and mortality with numerous solid malignancies is now well established, including but not limited to breast, endometrial, renal, colorectal, gallbladder, pancreatic, and gastric cancers [3–7]. Of note, the increased mortality may be attributable to comorbidities rather than disease-specific. A number of studies published in recent years have suggested that obesity raises the risk of ovarian carcinogenesis, but the association of obesity with death from ovarian cancer remains uncertain [3,8–10].

* Corresponding author at: 800 NE 10th Street, Suite 5040, Oklahoma City, OK 73104, United States.
E-mail addresses: camille-gunderson@ouhsc.edu (C.C. Gunderson), kai-ding@ouhsc.edu (K. Ding), justin-dvorak@ouhsc.edu (J. Dvorak), Kathleen-moore@ouhsc.edu (K.N. Moore), scott-mcmeekin@ouhsc.edu (D.S. McMeekin), doris-benbrook@ouhsc.edu (D.M. Benbrook).

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Obesity is a common generator of chronic inflammation as adipose tissue acts not only as an energy reservoir, but also an endocrine organ, leading to infiltration of activated immune cells and overproduction of pro-inflammatory cytokines. [11] A defining feature of the inflammatory microenvironment in adipose tissue is a marked increase in the amount of inflammatory macrophages, which release pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α). [12] Increased levels of CCL2, interleukin (IL)-4, IL-5, IL-6, IL-12, IL-13, C-reactive protein (CRP), TNF-α, and interferon-gamma are observed with obesity-induced chronic inflammation [13–15]. VEGF is a key regulator of tumor angiogenesis and is associated with obesity and poor prognosis with ovarian cancer and other solid tumors [16–18].

Existing epidemiologic evidence suggests that chronic inflammation is a mechanism in the development of ovarian cancer [19]. This is due to the dysregulated cytokine secretion which lends to excessive cell growth, malignant transformation, and survival of transformed cells [20,21]. Ness pioneered the concept that inflammatory factors may be involved in ovarian carcinogenesis in 1999 [22]. Since then, it has been demonstrated that the adnexal epithelium is chronically exposed to inflammation from the physiologic alterations that occur with ovulation and menstruation; pro-inflammatory cytokines which are normally present in ovulatory fluid and menstrual effluent are markedly elevated with ovarian cancer [23]. A number of inflammatory cytokines have been prospectively associated with development of ovarian cancer including IL-2, IL-4, IL-6, IL-12, and IL-13; this evidence suggests that inflammation is a pathway in epithelial ovarian carcinogenesis [20]. However, the contribution of cytokine-related inflammation to outcomes with ovarian cancer remains unclear.

The major determinant of prognosis with epithelial ovarian cancer is sensitivity to platinum-based chemotherapy, as 85% of women with advanced stage disease will experience disease recurrence [24]. Although most patients respond initially to chemotherapy, the emergence of drug resistance precludes adequate treatment, and in the face of platinum resistant disease, median survival is 12 months [25,26]. Undoubtedly, platinum sensitivity (or lack thereof) is the driver of prognosis with epithelial ovarian cancer. Limited data exists in the current literature evaluating the impact of obesity on the advent of platinum resistance. However, several pro-inflammatory cytokines have a prominent role in tumor progression, particularly IL-6. It enables metastasis via induction of tumor angiogenesis but also has a paracrine effect with co-regulation of other inflammatory cytokines such as TNF-α, CCL2, and VEGF [27,28]. Levels of IL-6 in ovarian cancer patients have been shown to correlate with chemoresistance in vitro and thus prognosis via activation of multiple signal transduction pathways [28,29].

Given the contribution of obesity to both chronic inflammation and ovarian carcinogenesis, we sought to determine whether obesity is associated with platinum resistance in women with high grade serous epithelial ovarian cancer.

2. Methods

An IRB-approved (study #3794) retrospective chart review was performed of patients who received primary treatment for epithelial ovarian cancer at The University of Oklahoma Health Sciences Center (Stephenson Cancer Center) from 1996 to 2013. All patients without high grade serous carcinoma of the ovary, fallopian tube, or peritoneum were excluded in order to homogenize results with respect to chemosensitivity, survival patterns, and serum markers, as these are known to differ by histology. Other exclusion criteria included refusal of chemotherapy, lack of available follow-up data regarding response to chemotherapy and survival, and absence of pre-operative serum available in the biospecimen repository for cytokine analysis. Patients with early stage (I, II) disease or those who received chemotherapy before PDS were also excluded. Body mass index (defined as weight in kilograms divided by the square of the height in meters [kg/m^2]) was measured at the time of diagnosis. Demographic, oncologic, and primary treatment characteristics were recorded. Although total number of chemotherapy lines was recorded, details of treatment type and number of cycles beyond primary therapy were not captured. Patients who underwent debulking surgery were classified as having no gross residual disease, residual disease <1 cm, or residual disease ≥1 cm.

Serum samples were assayed for the levels of cytokines using Bioplex Reagent Kit (Human Cytokine Group 1, 8-plex assay; Bio-Rad, CA) following the manufacturer's instructions. Briefly, magnetic beads coated with capture antibodies to the specific cytokines were added to the wells of a 96-well plate and the wells were washed twice. The serum samples were diluted four-fold with the assay kit diluent and placed in duplicate on the plate. Serially diluted standards and a blank were also placed on the plate in duplicate. The plate was then incubated for 30 min at room temperature with shaking. At the end of the incubation, the beads were washed three times and incubated with biotinylated detection antibodies for 30 min with shaking at room temperature. The beads were then washed three times and incubated with Streptavidin linked to a phycoerythrin fluorescent reporter for 10 min with shaking at room temperature. At the end of this incubation, the beads were washed three times and re-suspended in the assay buffer. The intensity of each fluorescent reporter in each well of the 96-well plate was measured using a plate reader (Bio-plex 200, Bio-Rad, CA). A standard curve was generated for each cytokine and used to determine the concentration of each cytokine in each well. Duplicate values were averaged for statistical analysis, and samples that had values too low to be detected were considered zero.

SAS version 9.3 (SAS Institute; Cary, NC) was used for all statistical analyses. Progression free survival (PFS) was defined as time from completion of primary treatment to time of recurrence. If disease did not recur, PFS was censored at the date of death or date of last follow-up. Platinum resistant disease was defined as disease recurrence in <-6 months, and platinum sensitive disease encompassed PFS ≥6 months. Platinum sensitivity was further categorized as platinum sensitive (PFS 6–12 months) and platinum super sensitive (PFS >-12 months). Overall survival (OS) was defined as time from date of diagnosis to date of death. If death did not occur within our study period, OS was censored at the date of last follow-up. When comparing serum cytokines to survival periods using the log-rank test, patients were dichotomized into 2 groups based on the median of the non-zero biomarker values. Median survival time was estimated by using the Kaplan-Meier survival estimator. The Wilcoxon rank-sum test and the Kruskal-Wallis tests (with Bonferroni-corrected p-values) were utilized to compare biomarker levels between 2 and 3 groups, respectively based on platinum resistant status. Association between 2 categorical variables was assessed by Chi-square test. Spearman's rank correlation coefficient was used to quantify the correlation between each of the serum cytokines and BMI due to skewed distribution of biomarker levels. Multivariate analyses of effect of cytokines were also performed using logistic regression (for platinum resistance status) or Cox regression (for PFS and OS). Factors considered in multivariate analyses include stage, residual disease, age, maintenance therapy, bevacizumab during primary treatment, and intraperitoneal chemotherapy. A two-sided p-value of <0.05 defines statistical significance.

3. Results

During the study period, a total of 672 patients received treatment of epithelial ovarian cancer at The University of Oklahoma. Of these, 86 patients met the following criteria: high grade serous histology, receipt of chemotherapy with adequate documentation of follow-up visits, and availability of banked pre-operative serum for analysis; these are the patients included herein. Table 1 demonstrates the subject characteristics. Median patient age was 63.7 years (range, 34–83). Most patients were Caucasian (88%) and non-diabetic (92%); all had advanced stage disease (III/IV). Of patients tested for genetic predisposition, 90% did not have a *BRCA* or Lynch syndrome mutation. All patients underwent