Published in final edited form as:

Cytokine. 2015 May ; 73(1): 108–113. doi:10.1016/j.cyto.2015.01.035.

Plasma immune analytes in patients with epithelial ovarian cancer

Matthew S. Block^{*,‡}, Matthew J. Maurer[†], Krista Goergen[†], Kimberly R. Kalli^{*}, Courtney L. Erskine[‡], Marshall D. Behrens[‡], Ann L. Oberg[†], and Keith L. Knutson^{**,‡}

^{*}Department of Oncology, Mayo Clinic, Rochester, MN

[†]Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN

[‡]Department of Immunology, Mayo Clinic, Rochester, MN

^{**}Vaccine and Gene Therapy Institute, Port Saint Lucie, FL

Abstract

Objectives—Inflammation is a common feature of epithelial ovarian cancer (EOC), and measurement of plasma markers of inflammation might identify candidate markers for use in screening or presurgical evaluation of patients with adnexal masses.

Methods—Plasma specimens from cohorts of 100 patients with advanced EOC (AJCC Stage III and IV), 50 patients with early stage EOC (Stage I and II), and 50 patients with benign surgical conditions were assayed for concentrations of multiple cytokines, tolllike receptor agonists, and vascular growth factors via ELISA and electrochemiluminescence. Immune proteins were then analyzed for association with EOC. Differences in plasma protein levels between benign, early, and advanced EOC patient groups were assessed with and without adjustment for plasma cancer antigen 125 (CA-125) levels.

Results—Out of 23 proteins tested, six—including interferon gamma (IFN γ), interleukin 6 (IL-6), IL-8, IL-10, tumor necrosis factor alpha (TNF α), and placental growth factor (PIGF)—were univariately associated with EOC (all $p < 0.005$), and one—IL-6—was associated with early stage EOC ($p < 0.0001$). Heat shock protein 90kDa beta member 1 (HSP90B1, gp96) was associated with EOC and early stage EOC with borderline statistical significance ($p = 0.039$ and $p = 0.026$, respectively). However, when adjusted for (CA-125), only HSP90B1 independently predicted EOC ($p = 0.008$), as well as early stage EOC ($p = 0.014$).

Conclusions—Multiple plasma cytokines, including IFN γ , IL-6, IL-8, IL-10, TNF α , PIGF, and HSP90B1 are associated with EOC. Of these, HSP90B1 is associated with EOC independent from the biomarker CA-125.

© 2015 Published by Elsevier Ltd.

Conflicts of Interest: None.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Epithelial ovarian cancer (EOC) is the fifth leading cause of cancer death in women and the leading cause of death among gynecologic cancers, with approximately 22,000 cases of EOC diagnosed annually and over 14,000 annual deaths [1]. The primary reason for the high lethality of EOC is that the majority of patients present with advanced (AJCC Stages III or IV) disease at the time of diagnosis, and cures for patients with regional or distant metastases are relatively uncommon. Patients with newly diagnosed EOC benefit from being triaged to a gynecologic oncologist for staging/cytoreductive surgery[2]; however, when current strategies are used to predict which patients have EOC as opposed to a benign adnexal mass, the error rate for non-invasive testing strategies is significant [3-5].

Blood-based biomarkers have been used to detect EOC since the discovery of cancer antigen 125 (CA-125) [6, 7]. CA-125, also known as MUC16, is a large extracellular mucin expressed by the majority of ovarian cancers as well as by epithelial cells in the female reproductive tract [8]. CA-125 is found on the surface of EOC cells and is shed into plasma. However, despite its widespread use as a diagnostic and therapeutic response marker, CA-125 has poor positive and negative predictive value when used as a biomarker for diagnosing the presence of EOC in women presenting with adnexal masses [9]. Human epididymis protein 4 (HE4), a secreted protease inhibitor expressed by EOC cells, has also been found at increased levels in patients with EOC [10], and the combination of CA-125 and HE4 has a higher specificity for EOC than does CA-125 alone when used to distinguish between malignant and benign masses [3]. As such, combining HE4 and CA-125 provides an improved means for diagnosing EOC in patients undergoing preoperative evaluation for adnexal masses [4, 5]. However, identification of additional plasma-based proteins capable of predicting EOC could significantly improve pre-diagnostic decision-making in patients with adnexal masses.

In the context of EOC, both HE4 and CA-125 are primarily produced by the malignant epithelial cells themselves. As such, they can only be detected once sufficient tumor is present to produce protein levels that can be detected in plasma. This requirement for a significant tumor burden may explain why tumor-derived analytes are unreliable for detecting EOC, as EOC often disseminates within the peritoneal cavity before forming a large tumor mass, and levels of HE4 and CA-125 may be normal even in the setting of advanced EOC.

Increasingly, the immune response to EOC has been shown to play a key role in modulating the disease. Tumor-infiltrating leukocytes (TILs) have been demonstrated in diagnostic EOC specimens and have great prognostic importance [11, 12]. Similarly, antibodies against EOC-derived antigens have been demonstrated in a subset of EOC patients and have also shown prognostic value [13]. Finally, a limited number of cytokines, including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF α), have been found at increased levels in EOC [14, 15]. Furthermore, other indicators of inflammation, including heat shock proteins and vascular growth factors, are elevated in plasma from a subset of EOC patients [16, 17]. As TILs are not identified until the time of surgery, and antibodies to any given antigen are only present in a subset (generally < 50%) of EOC patients, these indicators of immunity have

limited potential value in prediagnostic evaluation. However, the relative ease of detection of plasma cytokines and other inflammatory proteins makes them attractive as a possible means of early identification of EOC.

To assess the potential utility of multiple plasma-based inflammatory proteins in detecting EOC, we obtained presurgical plasma samples from a cohort of patients scheduled to undergo surgery for an adnexal mass. We then measured plasma concentrations of 20 cytokines, heat shock proteins, and vascular growth factors and assessed for differences in marker expression between patients with benign adnexal masses and patients with early or advanced stage EOC.

Materials and methods

Study Population

Frozen plasma specimens were obtained through the Mayo Clinic Biospecimen Repository for Ovarian Cancer Research (Mayo Clinic IRB #08-005749). Briefly, patients who were scheduled to undergo surgery for an adnexal mass were consented to provide presurgical plasma specimens. Patients were awake and fasting at the time of the blood draw, which took place prior to the start of surgery. For this retrospective study, 200 specimens were selected from women who were diagnosed with EOC of any stage, grade, and epithelial histology (serous, mucinous, endometrioid, clear cell, and mixed) between October 2002 and April 2008, or from women with benign conditions warranting diagnostic surgery during the same dates. Specimens were excluded if inadequate plasma was available, stage of cancer was unknown, age was greater than 82, or the patient experienced perioperative death (less than 90 days from surgery). Specimens were coded prior to assays and analysis such that all clinical information was provided without release of personal identifiers.

Biochemical Analysis of Plasma Proteins

Frozen plasma samples were stored at -80 °C until the time of use. Each sample underwent two freeze-thaw cycles prior to testing. Samples were assigned a random location for each assay plate. All assays were performed per the manufacturers' instructions using commercially available assays. Sample analysis was performed in duplicate. Enzyme-linked immunosorbent assays (ELISAs) were performed for heat shock protein 60 (hsp60, R&D Systems Inc., Minneapolis, MN), heparan sulfate (HS, LifeSpan Biosciences, Inc., Seattle, WA), Heparanase (HSPE, Biotang Inc., Waltham, MA), and heat shock protein 90kDa beta member 1 (hsp90B1 or gp96, Biotang Inc.). Electrochemiluminescence immunoassays (Meso Scale Discovery, Rockville, MD) were performed for interferon gamma (IFN γ), Interleukin-1 beta (IL-1 β), IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, C-X-C motif chemokine 10 (CXCL10 or IP-10), TNF α , CA-125, granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein 1 alpha (MIP-1 α), MIP-1 β , placental growth factor (PIGF), and vascular endothelial growth factor (VEGF).

Statistical Analysis

In initial quality control, all proteins were initially examined graphically to assess the performance of the assay in relation to the assay's limit of detection. Candidate biomarkers that were undetectable or had a substantial number of samples outside the limits of detection of the assay were excluded from further analyses (S1). The remaining analytes were log₂ transformed and median-adjusted to account for any plate effects. The distribution of analytes by patient group (benign vs. EOC (all stages) or benign vs. EOC (early stage)) was assessed graphically by jitter plots and Wilcoxon rank-sum tests. Logistic regression was used to assess association between biomarkers and patient groups; models were performed unadjusted as well as adjusted for CA-125 (CA-125 included as a covariate). Fourteen analytes passed quality control and were used in the final analyses; given the expected correlation among these markers a Bonferroni correction would be overly conservative, thus a p-value < 0.005 was used to determine significance.

Results

Patient characteristics

Cohorts of 50 patients with benign adnexal masses, 50 patients with early stage EOC (AJCC stage I or II [18]), and 100 patients with advanced stage EOC (stage III or IV) were selected (Table 1). The median ages for each cohort were 60.5 (benign—interquartile range 49-69), 54.5 (early stage EOC—interquartile range 50-69), and 63.0 (advanced stage EOC—interquartile range 54-69.5). The majority (81%) of advanced EOC cases were of high grade and serous histology, whereas serous, endometrioid, clear cell, and mixed histologies each represented a significant proportion of early stage tumors.

Univariate association of inflammatory biomarkers with EOC

Plasma levels of 23 candidate inflammatory biomarkers, along with CA-125, were assessed from pre-surgical blood specimens. Of these, nine markers were excluded from analysis due to many samples being at or below the lower limit of detection (S1). Univariate analysis was then performed on the remaining 14 markers to assess for differences in plasma marker concentrations between patients with benign adnexal masses versus EOC, and between patients with benign adnexal masses versus early stage EOC. Five biomarkers, including IL-6, IL-8, IL-10, TNF α , and PlGF, were found at significantly higher levels ($p < 0.005$) in EOC patients relative to patients with benign adnexal masses (Table 2, S2). One biomarker, IFN γ , was found at significantly lower levels in EOC patients relative to patients with benign adnexal masses (Table 2, S2). Of these, only IL-6 was significantly elevated in patients with early EOC relative to patients with benign adnexal masses. HSP90B1 was higher in EOC and early stage EOC with borderline statistical significance ($p = 0.039$ and $p = 0.026$, respectively).

Association after adjustment for CA-125

To assess which candidate biomarker(s) show potential to add discriminatory value to CA-125 in predicting the presence of EOC, logistic regression models were fit for each marker, adjusting for plasma CA-125 concentration (Table 3). After adjustment for CA-125,

HSP90B1 significantly predicted the presence of EOC (OR = 2.10, 95% CI: 1.22-3.88, $p = 0.008$) and early EOC (OR = 9.10, 95% CI: 1.96-64.36, $p = 0.014$). Levels of HSP90B1 and CA-125 were compared for each patient cohort (Figure 1).

Discussion

Inflammation is involved in the pathogenesis of many cancers, including EOC [19, 20]. We hypothesized that cytokines and other inflammatory biomarkers are identifiable in plasma from patients with EOC relative to patients with non-malignant adnexal masses. We demonstrated that plasma levels of IFN γ , IL-6, IL-8, IL-10, TNF α , and PIGF differ statistically between patients with versus without EOC, and that levels of IL-6 are higher in patients with early stage EOC relative to benign adnexal masses. While the six cytokines do not contribute independent discriminatory information after CA-125 adjustment, the heat shock protein HSP90B1 predicts the presence of EOC after CA-125 adjustment.

We have demonstrated that IL-6 plasma levels are significantly increased in EOC patients relative to patients with benign conditions. This is consistent with findings from other investigators [21, 22], although our study specifically notes that increased IL-6 levels are seen in early stage EOC. Median IL-6 levels were higher in patients with early EOC than patients with benign adnexal masses, and even higher in patients with advanced EOC than in patients with early EOC. However, due to significant overlap between IL-6 levels in the three cohort, plasma IL-6 is not reliable as a standalone biomarker for EOC. IL-6 is released under several non-malignant conditions, including infection, trauma, and exercise [23-25]. IL-6 secretion leads to increased acute phase protein production [26], neutrophil function [27], and B cell proliferation and differentiation [28]. In EOC, increased plasma IL-6 levels have been associated with poor outcomes [29], and IL-6 has been shown to promote EOC cell survival [30] and angiogenesis [31]. It is not completely clear which functions of IL-6 are most important for EOC pathogenesis, but a monoclonal antibody targeting IL-6 (siltuximab) has been tested as a therapeutic modality for recurrent EOC, with clinical benefit seen in a subset of patients [32].

While most studies of IL-6 biology have focused on membrane-bound IL-6 receptor, it has recently become increasingly apparent that the IL-6 receptor can be shed from the plasma membrane as soluble IL-6 receptor [33]. Soluble IL-6 receptor binds to the ubiquitously expressed membrane receptor gp130, and this can lead to trans-IL-6 signalling in many cell types. Soluble IL-6 receptor and gp130 have been found in elevated levels in serum and ascites from ovarian cancer patients [34, 35]. We are currently collecting serial plasma samples from EOC patients, and this cohort, once mature, should be able to address whether plasma levels of IL-6 and/or soluble IL-6 receptor could serve as a therapy response marker in EOC.

In addition to IL-6, we demonstrated increased plasma levels of IL-8, IL-10, TNF α , and PIGF in EOC patients relative to those with benign conditions, whereas IFN γ was noted to be at decreased levels. Circulating IL-8 [36], IL-10 [37], and TNF α [38] levels have been previously reported to be elevated in EOC patients relative to controls, whereas increased PIGF levels and decreased IFN γ levels have not been demonstrated to our knowledge. There

was no indication that IL-8, IL-10, TNF α , PlGF, or IFN γ are altered in early stage EOC relative to control patients.

HSP90B1 is a molecular chaperone with anti-apoptotic properties when expressed in EOC cells [39]. It has also been implicated in inflammatory responses due to its ability to stimulate toll-like receptor 4 (TLR-4) activation [40]. EOC expression of HSP90B1 has been assessed in ascites samples, and both nuclear and cytoplasmic expression of HSP90B1 can be identified [41]. To our knowledge, plasma levels of HSP90B1 have not previously been measured in EOC patients. We found that increased levels of HSP90B1 are seen in EOC patients (including early EOC patients) independent of CA-125 expression; however, given the significant overlap in HSP90B1 levels between patients with benign conditions versus EOC, plasma measurement of HSP90B1 is likely to add minimal value to CA-125 in screening or in prediagnostic assessment of patients.

Our studies demonstrate that inflammation—and in particular secretion of IL-6—occurs early in the pathogenesis of EOC. While our measurements of inflammatory markers were conducted on prediagnostic plasma samples to assess the utility of inflammatory molecules as disease-predictive biomarkers, it is likely that sampling of other sites, such as peritoneal fluid or vaginal secretions, would yield higher cytokine levels and a broader array of inflammatory markers. This may be useful in furthering our understanding of the immunopathogenesis of EOC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Mayo Clinic Ovarian Cancer SPORE, CA136393. The authors thank Candace Kostelec for assistance in preparing this manuscript.

References

1. Siegel R, Ward E, Brawley O, Jemal A. Cancer statistics, 2011. CA: A Cancer Journal for Clinicians. 2011; 61:212–236. [PubMed: 21685461]
2. Mayer AR, Chambers SK, Graves E, Holm C, Tseng PC, Nelson BE, Schwartz PE. Ovarian cancer staging: Does it require a gynecologic oncologist? Gynecologic Oncology. 1992; 47:223–227. [PubMed: 1468701]
3. Kadija S, Stefanovic A, Jeremic K, Radojevic MM, Nikolic L, Markovic I, Atanackovic J. The utility of human epididymal protein 4, cancer antigen 125, and risk for malignancy algorithm in ovarian cancer and endometriosis. International Journal of Gynecological Cancer. 2011 Publish Ahead of Print:10.1097/IGC.1090b1013e318234f318852.
4. Karlsen MA, Sandhu N, Hogdall C, Christensen IJ, Nedergaard L, Lundvall L, Engelholm SA, Pedersen AT, Hartwell D, Lydolph M, Laursen IA, Hogdall EV. Evaluation of he4, ca125, risk of ovarian malignancy algorithm (roma) and risk of malignancy index (rmi) as diagnostic tools of epithelial ovarian cancer in patients with a pelvic mass. Gynecologic Oncology. 2012; 127:379–383. [PubMed: 22835718]
5. Moore RG, Jabre-Raughley M, Brown AK, Robison KM, Miller MC, Allard WJ, Kurman RJ, Bast RC, Skates SJ. Comparison of a novel multiple marker assay vs the risk of malignancy index for the prediction of epithelial ovarian cancer in patients with a pelvic mass. Am J Obstet Gynecol. 2010; 203:228 e221–226. [PubMed: 20471625]

6. Canney PA, Moore M, Wilkinson PM, James RD. Ovarian-cancer antigen ca125 -a prospective clinical-assessment of its role as a tumor-marker. *Br J Cancer*. 1984; 50:765–769. [PubMed: 6208925]
7. Niloff JM, Knapp RC, Schaetzel E, Reynolds C, Bast RC. Ca125-antigen levels in obstetric and gynecologic patients. *Obstet Gynecol*. 1984; 64:703–707. [PubMed: 6208522]
8. Wang Y, Cheon DJ, Lu Z, Cunningham SL, Chen CM, Luo RZ, Xing D, Orsulic S, Bast RC Jr, Behringer RR. Muc16 expression during embryogenesis, in adult tissues, and ovarian cancer in the mouse. *Differentiation*. 2008; 76:1081–1092. [PubMed: 18637025]
9. Dodge JE, Covens AL, Lacchetti C, Elit LM, Le T, Devries-Aboud M, Fung-Kee-Fung M. Preoperative identification of a suspicious adnexal mass: A systematic review and meta-analysis. *Gynecologic Oncology*. 2012; 126:157–166. [PubMed: 22484399]
10. Hellstrom I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, Drescher C, Urban N, Hellstrom KE. The he4 (wfdc2) protein is a biomarker for ovarian carcinoma. *Cancer Res*. 2003; 63:3695–3700. [PubMed: 12839961]
11. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G. Intratumoral t cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med*. 2003; 348:203–213. [PubMed: 12529460]
12. Nelson BH. The impact of t-cell immunity on ovarian cancer outcomes. *Immunol Rev*. 2008; 222:101–116. [PubMed: 18363996]
13. Piura B, Piura E. Autoantibodies to tumor-associated antigens in epithelial ovarian carcinoma. *J Oncol*. 2009; 2009 581939.
14. Maccio A, Madeddu C. The role of interleukin-6 in the evolution of ovarian cancer: Clinical and prognostic implications--a review. *Journal of Molecular Medicine*. 2013; 91:1355–1368. [PubMed: 24057813]
15. Dobrzycka B, Terlikowski SJ, Kowalczyk O, Kinalski M. Circulating levels of tnfr-alpha and its soluble receptors in the plasma of patients with epithelial ovarian cancer. *Eur Cytokine Netw*. 2009; 20:131–134. [PubMed: 19825522]
16. Geisler JP, Geisler HE, Tammela J, Wiemann MC, Zhou Z, Miller GA, Crabtree W. Heat shock protein 27: An independent prognostic indicator of survival in patients with epithelial ovarian carcinoma. *Gynecologic Oncology*. 1998; 69:14–16. [PubMed: 9570992]
17. Lawicki S, Bedkowska GE, Gacuta-Szumarska E, Szmitkowski M. The plasma concentration of vegf, he4 and ca125 as a new biomarkers panel in different stages and sub-types of epithelial ovarian tumors. *J Ovarian Res*. 2013; 6:45. [PubMed: 23819707]
18. Edge SB.; Byrd DR.; Compton CC.; Fritz AG.; Greene FL.; Trotti, A. American joint committee on cancer (ajcc) cancer staging manual, 7th edition. 7. Chicago: Springer, Inc.; 2010.
19. Wu AH, Pearce CL, Tseng CC, Templeman C, Pike MC. Markers of inflammation and risk of ovarian cancer in los angeles county. *Int J Cancer*. 2009; 124:1409–1415. [PubMed: 19065661]
20. Toriola AT, Grankvist K, Agborsangaya CB, Lukanova A, Lehtinen M, Surcel HM. Changes in pre-diagnostic serum c-reactive protein concentrations and ovarian cancer risk: A longitudinal study. *Ann Oncol*. 2011; 22:1916–1921. [PubMed: 21292643]
21. Berek JS, Chung C, Kaldi K, Watson JM, Knox RM, Martinez-Maza O. Serum interleukin-6 levels correlate with disease status in patients with epithelial ovarian cancer. *Am J Obstet Gynecol*. 1991; 164:1038–1042. discussion 1042-1033. [PubMed: 2014824]
22. Scambia G, Testa U, Panici PB, Martucci R, Foti E, Petrini M, Amoroso M, Masciullo V, Peschle C, Mancuso S. Interleukin-6 serum levels in patients with gynecological tumors. *Int J Cancer*. 1994; 57:318–323. [PubMed: 8168990]
23. van der Poll T, Keogh CV, Guirao X, Buurman WA, Kopf M, Lowry SF. Interleukin-6 gene-deficient mice show impaired defense against pneumococcal pneumonia. *J Infect Dis*. 1997; 176:439–444. [PubMed: 9237710]
24. Pedersen BK, Febbraio MA. Muscle as an endocrine organ: Focus on muscle-derived interleukin-6. *Physiol Rev*. 2008; 88:1379–1406. [PubMed: 18923185]
25. Easton R, Balogh ZJ. Peri-operative changes in serum immune markers after trauma: A systematic review. *Injury*. 2014; 45:934–941. [PubMed: 24388280]

26. Bode JG, Albrecht U, Haussinger D, Heinrich PC, Schaper F. Hepatic acute phase proteins--regulation by il-6- and il-1-type cytokines involving stat3 and its crosstalk with nf-kappab-dependent signaling. *Eur J Cell Biol.* 2012; 91:496–505. [PubMed: 22093287]
27. Johnson JL, Moore EE, Tamura DY, Zallen G, Biffl WL, Silliman CC. Interleukin-6 augments neutrophil cytotoxic potential via selective enhancement of elastase release. *J Surg Res.* 1998; 76:91–94. [PubMed: 9695746]
28. Kopf M, Ramsay A, Brombacher F, Baumann H, Freer G, Galanos C, Gutierrez-Ramos JC, Kohler G. Pleiotropic defects of il-6-deficient mice including early hematopoiesis, t and b cell function, and acute phase responses. *Ann N Y Acad Sci.* 1995; 762:308–318. [PubMed: 7545368]
29. Scambia G, Testa U, Benedetti Panici P, Foti E, Martucci R, Gadducci A, Perillo A, Facchini V, Peschle C, Mancuso S. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. *Br J Cancer.* 1995; 71:354–356. [PubMed: 7841052]
30. Duan Z, Foster R, Bell DA, Mahoney J, Wolak K, Vaidya A, Hampel C, Lee H, Seiden MV. Signal transducers and activators of transcription 3 pathway activation in drug-resistant ovarian cancer. *Clin Cancer Res.* 2006; 12:5055–5063. [PubMed: 16951221]
31. Nilsson MB, Langley RR, Fidler IJ. Interleukin-6, secreted by human ovarian carcinoma cells, is a potent proangiogenic cytokine. *Cancer Res.* 2005; 65:10794–10800. [PubMed: 16322225]
32. Coward J, Kulbe H, Chakravarty P, Leader D, Vassileva V, Leinster DA, Thompson R, Schioppa T, Nemeth J, Vermeulen J, Singh N, Avril N, Cummings J, Rexhepaj E, Jirstrom K, Gallagher WM, Brennan DJ, McNeish IA, Balkwill FR. Interleukin-6 as a therapeutic target in human ovarian cancer. *Clin Cancer Res.* 2011; 17:6083–6096. [PubMed: 21795409]
33. Wolf J, Rose-John S, Garbers C. Interleukin-6 and its receptors: A highly regulated and dynamic system. *Cytokine.* 2014; 70:11–20. [PubMed: 24986424]
34. Kovacs E. Investigation of interleukin-6 (il-6), soluble il-6 receptor (sil-6r) and soluble gp130 (sgp130) in sera of cancer patients. *Biomed Pharmacother.* 2001; 55:391–396. [PubMed: 11669502]
35. Lo CW, Chen MW, Hsiao M, Wang S, Chen CA, Hsiao SM, Chang JS, Lai TC, Rose-John S, Kuo ML, Wei LH. Il-6 trans-signaling in formation and progression of malignant ascites in ovarian cancer. *Cancer Res.* 2011; 71:424–434. [PubMed: 21123455]
36. Aune G, Stunes AK, Lian AM, Reseland JE, Tingulstad S, Torp SH, Syversen U. Circulating interleukin-8 and plasminogen activator inhibitor-1 are increased in women with ovarian carcinoma. *Results Immunol.* 2012; 2:190–195. [PubMed: 24371583]
37. Wertel I, Polak G, Tarkowski R, Kotarska M. Evaluation of il-10 and tgf-beta levels and myeloid and lymphoid dendritic cells in ovarian cancer patients. *Ginekol Pol.* 2011; 82:414–420. [PubMed: 21853929]
38. Gadducci A, Ferdeghini M, Castellani C, Annicchiarico C, Galletti O, Prontera C, Bianchi R, Facchini V. Serum levels of tumor necrosis factor (tnf), soluble receptors for tnf (55- and 75-kda stnfr), and soluble cd14 (scd14) in epithelial ovarian cancer. *Gynecologic Oncology.* 1995; 58:184–188. [PubMed: 7542621]
39. Kim YJ, Lee SA, Myung SC, Kim W, Lee CS. Radicicol, an inhibitor of hsp90, enhances trail-induced apoptosis in human epithelial ovarian carcinoma cells by promoting activation of apoptosis-related proteins. *Mol Cell Biochem.* 2012; 359:33–43. [PubMed: 21800052]
40. Thuringer D, Hammann A, Benikhlef N, Fourmaux E, Bouchot A, Wettstein G, Solary E, Garrido C. Transactivation of the epidermal growth factor receptor by heat shock protein 90 via toll-like receptor 4 contributes to the migration of glioblastoma cells. *J Biol Chem.* 2011; 286:3418–3428. [PubMed: 21127066]
41. Elstrand MB, Stavnes HT, Trope CG, Davidson B. Heat shock protein 90 is a putative therapeutic target in patients with recurrent advanced-stage ovarian carcinoma with serous effusions. *Hum Pathol.* 2012; 43:529–535. [PubMed: 21864883]

Highlights

- Plasma IFN γ , IL6, IL8, IL10, TNF α , PlGF and HSP90B1 associate with ovarian cancer.
- IL-6 levels are associated with early stage ovarian cancer.
- HSP90B1 is independently associated with ovarian cancer after adjustment for CA125.

Figure 1.

CA-125 and HSP90B1 levels are compared for each control, early EOC, and advanced EOC patient. The dashed line represents the institutional upper limit of normal for CA-125 (35 units/ml).

Table 1**Patient characteristics**

Clinical characteristics of each cohort of patients are shown.

	Benign (N=50)	Early Stage (N=50)	Advanced Stage (N=100)	Total (N=200)
Age at diagnosis				
Median	60.5	54.5	63.0	59.5
Q1, Q3	49.0, 69.0	50.0, 69.0	54.0, 69.5	52.0, 69.0
Range	(38.0-82.0)	(35.0-82.0)	(36.0-81.0)	(35.0-82.0)
Race				
White	47 (94.0%)	41 (82.0%)	89 (89.0%)	177 (88.5%)
Asian	0 (0.0%)	1 (2.0%)	0 (0.0%)	1 (0.5%)
Other	0 (0.0%)	0 (0.0%)	1 (1.0%)	1 (0.5%)
Choose Not to Disclose	0 (0.0%)	0 (0.0%)	1 (1.0%)	1 (0.5%)
Unknown	3 (6.0%)	8 (16.0%)	9 (9.0%)	20 (10.0%)
Ethnicity				
Not Hispanic or Latino	37 (74.0%)	29 (58.0%)	65 (65.0%)	131 (65.5%)
Choose Not to Disclose	2 (4.0%)	0 (0.0%)	1 (1.0%)	3 (1.5%)
Unknown	11 (22.0%)	21 (42.0%)	34 (34.0%)	66 (33.0%)
CA-125 (units/ml)				
Median	10.4	160.1	1043.9	219.8
Q1, Q3	8.0, 17.1	43.8, 735.8	354.2, 3220.8	22.1, 1552.5
Range	(5.1-552.7)	(7.9-7264.8)	(16.3-19131.6)	(5.1-19131.6)
Histologic type				
Serous	0 (0.0%)	14 (28.0%)	81 (81.0%)	95 (47.5%)
Mucinous	0 (0.0%)	6 (12.0%)	0 (0.0%)	6 (3.0%)
Endometrioid	0 (0.0%)	13 (26.0%)	9 (9.0%)	22 (11.0%)
Clear cell	0 (0.0%)	9 (18.0%)	4 (4.0%)	13 (6.5%)
Mixed Epithelial	0 (0.0%)	8 (16.0%)	6 (6.0%)	14 (7.0%)
Benign	50 (100.0%)	0 (0.0%)	0 (0.0%)	50 (25.0%)
Ascites				
Yes	NA	12 (24.0%)	59 (59.0%)	71 (47.3%)
No	NA	34 (68.0%)	23 (23.0%)	57 (38.0%)
Unknown	NA	4 (8.0%)	18 (18.0%)	22 (14.7%)
Debulking				
Optimal	NA	49 (98.0%)	88 (88.0%)	137 (91.3%)
Sub-optimal	NA	0 (0.0%)	9 (9.0%)	9 (6.0%)
Unknown	NA	1 (2.0%)	3 (3.0%)	4 (2.7%)
Stage				
I	NA	36 (72.0%)	0 (0.0%)	36 (24.0%)
II	NA	14 (28.0%)	0 (0.0%)	14 (9.3%)

	Benign (N=50)	Early Stage (N=50)	Advanced Stage (N=100)	Total (N=200)
III	NA	0 (0.0%)	83 (83.0%)	83 (55.3%)
IV	NA	0 (0.0%)	17 (17.0%)	17 (11.3%)
Grade				
Missing	NA	1	3	4
Low	NA	16 (32.7%)	7 (7.2%)	23 (15.8%)
High	NA	33 (67.4%)	90 (92.8%)	123 (84.2%)

Table 2

Univariate modeling of marker associations with epithelial ovarian cancer Wilcoxon Rank Sum tests were performed to assess for differences in the distributions of each cohort (benign versus early EOC and benign versus any EOC).

Variable	Benign		Early Stage		High Stage		Benign vs. Early Stage		Benign vs. Cancer (either stage)	
	Median	IQR	Median	IQR	Median	IQR	Wilcoxon p-value	Wilcoxon p-value	Wilcoxon p-value	Wilcoxon p-value
TLR Ligands										
Heparan sulfate	90.41	(68.37, 114.79)	95.34	(64.77, 114.98)	87.41	(67.22, 116.41)	0.58839	0.58839	0.71273	0.71273
HSP60	1.16	(0.09, 2.48)	1.03	(0.27, 3.30)	1.33	(0.32, 3.82)	0.84641	0.84641	0.49057	0.49057
HSP90B1	85.32	(73.21, 104.31)	98.52	(85.13, 117.63)	98.57	(75.10, 123.57)	0.02620	0.02620	0.03850	0.03850
Cytokines										
IFN γ	0.36	(0.19, 2.12)	0.29	(0.08, 1.11)	0.18	(0.03, 0.45)	0.11832	0.11832	0.00216	0.00216
IL-2	0.29	(0.13, 0.43)	0.24	(0.12, 0.31)	0.24	(0.13, 0.38)	0.47544	0.47544	0.64558	0.64558
IL-6	0.26	(0.16, 0.42)	0.78	(0.36, 3.98)	1.30	(0.56, 3.32)	<0.0001	<0.0001	<0.0001	<0.0001
IL-8	0.44	(0.26, 1.33)	0.74	(0.45, 1.32)	0.99	(0.51, 2.37)	0.05106	0.05106	0.00173	0.00173
IL-10	0.33	(0.11, 0.58)	0.36	(0.22, 0.67)	0.65	(0.36, 1.10)	0.21085	0.21085	0.00053	0.00053
IP-10	90.06	(62.63, 137.34)	78.33	(46.02, 120.91)	136.86	(67.30, 258.75)	0.48842	0.48842	0.08601	0.08601
MIP-1 α	4.37	(2.77, 7.05)	4.24	(2.04, 7.18)	6.21	(3.04, 9.10)	0.98898	0.98898	0.08071	0.08071
MIP-1 β	39.58	(23.81, 53.64)	35.38	(28.42, 52.11)	47.39	(33.46, 80.27)	0.82809	0.82809	0.03519	0.03519
TNF α	2.92	(0.37, 4.01)	3.26	(2.38, 4.45)	4.63	(3.29, 6.73)	0.18222	0.18222	0.00008	0.00008
Vascular Growth Factors										
PIGF	14.84	(11.04, 17.83)	18.18	(11.93, 23.34)	19.27	(15.81, 23.09)	0.01659	0.01659	0.00007	0.00007
VEGF	47.07	(18.41, 82.60)	46.92	(22.06, 87.15)	63.42	(40.04, 158.82)	0.63185	0.63185	0.02305	0.02305

Table 3

Modeling of marker associations with versus without adjustment for CA-125 Logistic regression was performed with and without adjustment for the known EOC biomarker CA-125 to assess whether observed variations between benign and EOC cohorts are dependent on CA-125 levels.

Variable	Logistic OR	Unadjusted Logistic 95% CI	Benign vs. Early Stage		CA-125 Adjusted Logistic 95% CI
			Logistic P-Value	Logistic OR	
TLR Ligands					
Heparan sulfate	0.887	(0.563, 1.277)	0.53638	1.154	(0.596, 1.809)
HSP60	1.112	(0.819, 1.528)	0.49711	1.500	(0.97, 2.408)
HSP90B1	2.551	(1.296, 6.245)	0.02019	9.099	(1.962, 64.358)
Cytokines					
IFN γ	0.714	(0.435, 1.145)	0.16912	0.586	(0.244, 1.267)
IL-2	0.945	(0.602, 1.387)	0.76324	0.838	(0.476, 1.302)
IL-6	4.500	(2.235, 11.627)	0.00027	1.709	(0.673, 5.412)
IL-8	1.049	(0.707, 1.569)	0.81093	1.016	(0.568, 1.739)
IL-10	1.244	(0.721, 2.384)	0.45096	0.983	(0.327, 2.133)
IP-10	0.884	(0.587, 1.321)	0.54925	0.638	(0.311, 1.219)
MIP-1 α	1.033	(0.725, 1.474)	0.85683	0.893	(0.529, 1.519)
MIP-1 β	1.085	(0.645, 1.837)	0.75760	0.976	(0.454, 2.082)
TNF α	1.397	(0.924, 2.151)	0.11823	1.518	(0.812, 3.026)
Vascular Growth Factors					
PIGF	2.595	(1.237, 5.846)	0.01527	2.167	(0.662, 7.908)
VEGF	1.095	(0.864, 1.397)	0.45533	0.842	(0.579, 1.2)