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Plasma immune analytes in patients with epithelial ovarian cancer

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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, toll-like 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 (IFNγ), interleukin 6 (IL-6), IL-8, IL-10, tumor necrosis factor alpha (TNFα), and placental growth factor (PlGF)—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 90 kDa 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 IFNγ, IL-6, IL-8, IL-10, TNFα, PlGF, and HSP90B1 are associated with EOC. Of these, HSP90B1 is associated with EOC independent from the biomarker CA-125.

1. 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 Stage 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.

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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 (TNFα), 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 pre-diagnostic 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.

2. Materials and methods

2.1. 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.

2.2. 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 90 kDa beta member 1 (hsp90B1 or gp96, Biotang Inc.). Electrochemiluminescence immunoassays (Meso Scale Discovery, Rockville, MD) were performed for interferon gamma (IFNγ), Interleukin-1 beta (IL-1β), 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), TNFα, CA-125, granulocyte macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein 1 alpha (MIP-1α), MIP-1β, placental growth factor (PlGF), and vascular endothelial growth factor (VEGF).

2.3. 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 versus EOC (all stages) or benign versus 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.

3. Results

3.1. 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.

3.2. 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, TNFα, 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, IFNγ, 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 rela-