Urinary interleukin-1β levels among gynecological patients

Kamisha T Woolery, Mitchel S Hoffman, Joshua Kraft, Santo V Nicosia, Ambuj Kumar and Patricia A Kruk

Abstract

Background: Early detection of epithelial ovarian cancer (OC) is necessary to overcome the high mortality rate of late stage diagnosis; and, examining the molecular changes that occur at early disease onset may provide new strategies for OC detection. Since the deregulation of inflammatory mediators can contribute to OC development, the purpose of this pilot study was to determine whether elevated urinary levels of Interleukin-1beta (IL-1 beta) are associated with OC and associated clinical parameters.

Methods: Urinary and serum levels of IL-1 beta were analyzed by ELISA from a patient cohort consisting of healthy women (N = 10), women with ovarian benign disease (N = 23), women with OC (N = 32), women with other benign gynecological conditions (N = 22), and women with other gynecological cancers (N = 6).

Results: Average urinary IL-1 beta levels tended to be elevated in ovarian benign (1.26 pg/ml) and OC (1.57 pg/ml) patient samples compared to healthy individuals (0.36 pg/ml). Among patients with benign disease, urinary IL-1β levels were statistically higher in patients with benign inflammatory gynecologic disease compared to patients with non-inflammatory benign disease. Interestingly, urinary IL-1 beta levels tended to be 3-6x greater in patients with benign ovarian disease or OC as well as with a concomitant family history of ovarian and/or breast cancer compared to similar patients without a family history of ovarian and/or breast cancer. Lastly, there was a pattern of increased urinary IL-1 beta with increasing body mass index (BMI); patients with a normal BMI averaged urinary IL-1 beta levels of 0.92 pg/ml, overweight BMI averaged urinary IL-1 beta levels of 1.72 pg/ml, and obese BMI averaged urinary IL-1 beta levels of 5.26 pg/ml.

Conclusions: This pilot study revealed that urinary levels of IL-1 beta are elevated in patients with epithelial OC supporting the thought that inflammation might be associated with cancer progression. Consequently, further studies of urinary IL-1 beta and the identification of an inflammatory profile specific to OC development may be beneficial to reduce the mortality associated with this disease.

Keywords: Interleukin-1 beta, Obesity, Ovarian cancer, Urine, Body mass index
sonography can result in false positive results and unnecessary surgery [7]. Unfortunately, utilizing these methods either alone or in combination does not produce the desired results for early disease detection. Therefore, examining the molecular changes that occur at early disease onset may provide new approaches or biomarkers for OC detection.

Deregulation of inflammation due to overexpression of proinflammatory proteins contributes to the malignant phenotype by supporting cancer cell growth and tumor invasion. Since the proinflammatory cytokine, interleukin-1β (IL-1β), is constitutively expressed in OC [8] and elevated in serum of OC patients [9], in this pilot study, we sought to assess whether elevated urinary levels of IL-1β are associated with OC and related clinical parameters.

**Methods**

**Patient cohort**

With prior University of South Florida Institutional Review Board committee approval for study # 4739, urine and serum samples were collected from an initial cohort of healthy controls (N = 7), women with ovarian benign disorders (N = 12), and patients with epithelial OC (N = 20) at the H. Lee Moffitt Cancer Center (MCC). The second urine and serum cohort collected with University of South Florida Institutional Review Board committee approval for study # Pro00003119 at the University of South Florida (USF) consisted of healthy controls (N = 3), women with ovarian benign disease (N = 11), women with epithelial OC (N = 12), women with other benign gynecologic disease (N = 22), and other gynecologic cancers (N = 6). The OC category, diagnosed at time of initial cytoreductive surgery, consisted of women diagnosed with OC and primary peritoneal cancer, which is often related to OC. The samples collected from women with ovarian benign disease consisted of a broad range of non-malignant gynecologic disorders. Though these 93 samples comprise a small pilot study (Table 1), they are representative of a typical clinical practice with regards to histological distribution. Table 1 shows an age matched subset (N = 87) developed from the OC patients in the USF + MCC cohorts to produce an OC patient group with an age range (26–75 years) within two standard deviations of mean age to match healthy control (37–60 years) and ovarian benign disease (28–81) groups, respectively. Where possible, H & E sections from paraffin blocks were reviewed to confirm the histologic diagnosis according to International Federation of Gynecology and Obstetrics (FIGO) scores. H & E sections were also reviewed among patients with benign disease to distinguish between patients with inflammatory disease (including endometriosis, pelvic inflammatory disease) and non-inflammatory disease (including cystadenoma, leiomyoma, ovarian cysts). Anonymized information regarding patient age, body mass index (BMI), and tumor type were also obtained as per availability.

**Table 1 Histologic diagnoses and clinical characteristics of the study cohort**

| Category                                      | USF + MCC cohort | Other benign gynecological conditions (22) | Other gynecological cancers (6) | Ovarian cancer (32) | Age match (26) |
|-----------------------------------------------|------------------|-------------------------------------------|---------------------------------|--------------------|---------------|
| Age (mean ± SD)                               | 49.3 ± 10.3      | 42.59 ± 9.5                               | 59.17 ± 8.2                     | 60.91 ± 15.6       | 57.7 ± 15.0   |
| Age (range)                                   | 37-66            | 30-62                                     | 20-77                           | 26-92              | 26-78         |

**Sample preparation**

Urine and serum samples were collected from patients, anonymized to protect patient identity, and released from the tissue banks for this research project. All samples were kept on ice following collection. MCC urine samples were treated with a standard protease inhibitor cocktail (80 ug/mL 4-(2-aminoethyl)-benzene sulfonyle fluoride, 200 ug/mL ethylenediaminetetraacetic acid (EDTA), 0.2 ug/mL leupeptin, 0.2 ug/mL pepstatin, Sigma Scientific, St. Louis, MI). All urine samples were centrifuged at 3000 x g. Urinary supernates and serum samples were then aliquoted and stored at −20°C.

**Enzyme-linked immunosorbant assay**

To measure IL-1β levels in patients’ urine and serum, samples were assayed using the quantitative sandwich enzyme-linked immunosorbant assay (ELISA; R&D Systems, Inc., Minneapolis, MN) according to the manufacturer’s instructions. Fluorescence was read on an ELx800 Absorbance Microplate Reader (Biotek, Winooski, Vermont) using Gen5 Data Analysis Software (Biotek, Winooski, Vermont).
Figure 1 (See legend on next page.)
Resultant values were derived from a standard curve and expressed as the mean IL-1β concentration of triplicate samples ± standard error (S.E.).

**Statistical analysis**

Samples for IL-1β ELISA were run in triplicate and the data subjected to descriptive, parametric, Kruskal-Wallis, Mann–Whitney U, Spearman correlation, and Wilcoxon W analyses.

**Results**

**Urinary levels of IL-1β generally decrease with increasing patient age**

Levels of urinary IL-1β were compared in 93 patients of the entire cohort and 87 patients of the age matched subset. Though this cohort comprises a small pilot study, it is representative of our institutional clinical practice in regard to OC histology and stage distribution as well as in keeping with other comparable pilot studies. Average urinary IL-1β levels were compared to patient age and as divided by decade grouping. The highest levels of urinary IL-1β were found in the 20–29 and 30–39 age groups in both the entire cohort (Figure 1A) and the age matched subset (Figure 1B), respectively, suggesting an inverse relationship between increasing age and urinary IL-1β levels. Additionally, levels of serum IL-1β were compared in 19 patients of the entire cohort. In agreement with comparisons between urinary IL-1β levels and age, the highest average serum IL-1β level was detected in the 20–29 age group (Figure 1C), further suggesting a possible inverse correlation between age and IL-1β levels. Despite the tendency for increased urinary IL-1β levels in the 20–29 and 30–39 age groups, no statistical differences were found.

**Urinary IL-1β levels appear elevated in patients with ovarian cancer**

The levels of urinary IL-1β were generally negligible (average 0.36 pg/ml) in healthy control samples of the entire cohort and the age matched subset, respectively (Figure 2A and B). However, urinary IL-1β levels from women with benign ovarian disease were approximately 4-fold higher compared to healthy controls (average benign 1.26 pg/ml) (Figure 2A and B). Urinary levels of IL-1β from OC patients exhibited the highest average levels of 1.57 pg/ml IL-1β in the entire cohort (Figure 2A) and 1.88 pg/ml IL-1β in the age matched subset (Figure 2B), respectively. Further, levels of urinary IL-1β in non-ovarian benign gynecological disorders (average 1.56 pg/ml) and in non-ovarian gynecological cancers (average 1.36 pg/ml) were statistically increased compared to healthy controls (p ≤0.005 and 0.05, respectively) (Figure 2A and B). Lastly, when urinary IL-1β levels were compared among patients with benign disease, but differentiated on the basis of noted inflammatory involvement (including endometriosis, pelvic inflammatory disease), average urinary IL-1β levels were statistically increased in inflammatory benign disorders (1.88 pg/ml) compared to non-inflammatory benign disorders (0.82 pg/ml) (p =0.001) (Figure 2C).

When urinary IL-1β levels were compiled in the age matched cohort with respect to diagnosis and age, the average age in the healthy controls, benign ovarian disorders, and OC patients was 49.3, 52.2, and 57.7 years, respectively (Figure 3A). The average urinary levels of IL-1β in these healthy controls, benign ovarian disorders, and OC patients was 0.36 pg/ml, 1.30 pg/ml, and 1.88 pg/ml, respectively (Figure 3A) supporting a trend for increased urinary IL-1β levels with disease progression.

Levels of corresponding urine and serum IL-1β were available from 19 patients. Compared to controls, paired serum IL-1β showed a similar pattern of increased average IL-1β levels in the benign ovarian and OC group, respectively, with the highest average levels in the OC group (Figure 3B). Since there was a significant Spearman correlation between serum and urinary IL-1β levels in ovarian benign disorders (r = 0.949, N = 4, p = 0.051) and in ovarian cancers (r = 0.724, N = 11, p = 0.012), and there was a very limited number of available serum samples, all remaining clinical comparisons were completed in urine samples only.

**Urinary IL-1β levels are increased in patients with a family history of cancer**

Using the age matched cohort subset, urinary IL-1β levels were analyzed with respect to a family history of cancer though the BRCA1 status of these patients could not be confirmed. While the number of specimens was small, in a patient with benign ovarian disease and no known family history of cancer, urinary IL-1β levels were 0.33 pg/ml; while average levels were 0.96 pg/ml in five patients with benign ovarian disease and a family history of cancer (Figure 4A). Though we could not identify the lack of family history of cancer in most of the OC patients, in those OC patients with confirmed
Figure 2 (See legend on next page.)
family history of cancer, average urinary IL-1β levels were 6.33 pg/ml (Figure 4A), while the average urinary IL-1β levels of the remaining OC patients was 1.16 pg/ml. However, when sample 3119–13 which was about 20-fold higher than other samples in the OC group was excluded from the analyses, the average urinary IL-1β levels decreased to 1.01 pg/ml (Figure 4A).

Thirteen patient samples of the age matched subset were further narrowed to 8 patients with a first degree family history of ovarian and/or breast cancer. In two patients with benign ovarian disease and a family history of ovarian and/or breast cancer, average urinary IL-1β levels were three times higher (0.99 pg/ml) compared to patients with benign ovarian disease, but without a family history of ovarian and/or breast cancer (0.33 pg/ml). Likewise, among three patients with OC and a family history of ovarian and/or breast cancer, average urinary IL-1β levels were elevated (6.33 pg/ml) compared to OC patients without a family history of ovarian and/or breast cancer (1.16 pg/ml) (Figure 4B).

BMI classification (31.94) and demonstrated a 4-fold higher average urinary IL-1β level of 2.66 pg/ml (Figure 5B) compared to the healthy controls. OC patients had an average overweight BMI of 29.13 and 4.7-fold higher average urinary IL-1β level of 3.15 pg/ml (Figure 5B) compared to the control group. While these comparisons failed to achieve statistical significance, overall, our data suggest a trend of increased urinary IL-1β levels with increasing BMI.

**Discussion**

While inflammation is an essential biological process for normal development and tissue homeostasis, it is also involved in a number of pathologic conditions including tissue injury, chronic inflammation, immunological diseases, and cancer [11]. Epidemiological studies have shown a link between chronic inflammation and risk for cancer as evidenced by prolonged infection with Helicobacter pylori and gastric cancer, inflammatory bowel disease and colon cancer, and prostatitis and prostate cancer [12]. In the ovary, chronic inflammation resulting from repeated ovulatory wounding and repair promotes oxidative stress which enhances DNA replication errors and ultimately, oncogenesis [13]. Inflammation is regulated by several factors that can either promote or inhibit inflammation and since epithelial OCs are highly inflammatory, this pilot study evaluated urinary levels of the pro-inflammatory mediator, IL-1β against clinical parameters in order to gain a better understanding of this disease. This study was able to evaluate four clinical parameters in relation to urinary IL-1β levels: (1) patient age, (2) diagnosis, (3) family history of cancer, and (4) BMI.

When we considered patient age at the time of sample collection, the highest levels of urinary IL-1β levels were found in the 20–29 and 30–39 years age groups followed by declining IL-1β levels as age increased. In keeping with our findings and according to the American Society for Reproductive Medicine, a woman’s potential reproductive capacity begins to gradually decline at approximately 32 years of age and more rapidly decreases after 37 years of age [14]. IL-1β has been suggested to play a role in female reproduction; specifically in ovulation and oocyte maturation, and inflammatory-linked mechanisms, such as production and activation of proteolytic enzymes, prostaglandin production, nitric oxide production, cellular metabolism, and steroidogenesis [15,16]. Therefore, it seems likely that after 40 years of age, there
Figure 3 (See legend on next page.)
would be a decrease in urinary IL-1β levels as oocyte maturation and ovulation decrease in preparation for menopause. In contrast, Vural, et al. found higher plasma levels of IL-1β in postmenopausal (≥48.6 years) women than in premenopausal (30.5 ± 2.5 years) women with levels of IL-1β decreasing below the premenopausal levels only after hormone replacement therapy [17]. Therefore, it is possible that the sharp decrease in urinary IL-1β levels seen in our study in women >60 years may be due, in part, to the usage of hormone replacement therapy; however, this clinical information was not available for confirmation.

Urinary IL-1β levels alone had limited success in differentiating disease status. IL-1β is present in the serum and ascites of OC patients [9] and has been shown to be involved with cancer tumorigenesis, angiogenesis, and metastasis [18]. The inability of urinary IL-1β to differentiate between benign and malignancy may be confounded by the inflammatory nature of so many benign and cancer conditions. That is, many benign ovarian conditions develop in an inflammatory microenvironment. For instance, endometrioma, a form of endometriosis in the ovary, is a highly inflammatory condition [19] which would expectedly result in high levels of urinary IL-1β [20]. Proinflammatory markers including serum C-reactive protein, IL-6, and IL-8 have all been used in clinical studies in an attempt to differentiate between normal, benign tumor, and OC [21-23]. Immunohistochemical analyses have shown differential expression of IL-18 and its receptors in benign ovarian tumors, borderline ovarian tumors, and ovarian carcinomas [24]. Therefore, developing and employing a panel of inflammatory mediators, including urinary IL-1β, may eventually benefit differential diagnostic and prognostic outcomes of OC.

Patient samples with confirmed family history of cancer were limited in this small pilot study. Nonetheless, urinary IL-1β levels tended to be highest in patients with benign ovarian disease and a (first degree) family history of breast and/or ovarian cancer compared to patients with benign ovarian disease, but without a family history of breast and/or ovarian cancer. Likewise, elevated levels of urinary IL-1β were found among OC patients with a family history of breast and/or ovarian cancer compared to OC patients from families without a family history of disease. This supports the recent dualistic model that epithelial OCs arise as either two types: Type 1 and Type 2 [25]. Type 1 tumors may arise in a step-wise progression from a benign precursor lesion such as, the highly inflammatory condition, endometriosis. However, it is important to remember that not all individuals at risk for OC develop the disease so that secondary events, perhaps beyond a family history, may be necessary to promote disease.

Obesity as a risk factor of OC remains controversial. Recently, a meta-analysis of 47 epidemiological studies found increased OC risk with high BMI [26]. The Ovarian Cancer Association Consortium investigated 15 case–control studies and found overweight and obese women were associated with increased risk of OC [27]. The National Institutes of Health also found that BMI was significantly associated with increased OC risk in women who never used hormone therapy [28]. Canchola, et al. found a positive association between OC risk and adult weight gain, waist circumference, and waist-to-hip ratio, but no association to overall obesity as classified by BMI [29]. In agreement, Delort, et. al. also noted high waist-to-hip ratio associated with increased risk of OC though they found no association with BMI [30]. In contrast, Schouten, et al. reported no overall association between BMI and risk of OC; however, they did report a positive association with high BMI and increased OC risk among premenopausal women [31]. More recent prospective studies reported no significant relationship between BMI and OC risk, irrespective of menopausal status [32,33]. Likewise, evidence reported by McGee, et. al. does not support a risk for OC with weight or weight gain among BRCA1 or BRCA2 mutation carriers [34]. Interestingly, Engeland et al. also reported that the risk for OC was not associated with adult BMI, but suggested a possible increased risk in women who were obese in young adulthood [35].

In this pilot study, one of the most apparent clinical features related to elevated urinary IL-1β was BMI. We found increased urinary IL-1β levels associated with higher BMI. Overweight and obese patients were most likely to be diagnosed with OC and ovarian benign disorders, respectively, while concomitantly demonstrating the highest average urinary IL-1β levels. In contrast, healthy controls with normal BMI exhibited the lowest average urinary IL-1β. Among our data was a single patient case classified as underweight, but with elevated urinary IL-1β levels and a diagnosis of OC. It is tempting to speculate that this individual may have had cachexia at the time of sample collection where weight loss due
Figure 4 Urinary IL-1β levels increase with familial history of cancer. Urinary IL-1β was analyzed in triplicate by ELISA and data expressed as mean (pg/ml) ± standard error in age matched subset cohort following descriptive, Mann–Whitney U and Wilcoxon W analyses. (A) Patients with family history of any cancer. (B) Patients with family history of ovarian and/or breast cancer in first degree family members only.
Figure 5 (See legend on next page.)
to the increased glucose, lipid, and protein requirements of the tumor [36] could manifest as low BMI compounded with elevated urinary IL-1β as a result of advanced disease.

Obesity and elevated IL-1β levels in OC patients may contribute to OC mortality. While some studies have shown no association between BMI five to ten years prior to OC diagnosis and OC mortality [37,38], they do suggest that obesity is associated with poor outcome [39]. Consequently, obesity itself may not be the leading factor for increased OC mortality, but may act as a comorbidity factor. For instance, difficulty of proper chemotherapy dosages for obese patients may contribute to poor disease outcome. A study of dosing practices of clinicians found that a significant proportion of OC patients with advanced disease were overweight or obese, as seen in the current study, and under-dosing in obese populations was common [40]. The variability in dosing when prescribing chemotherapy is largely due to concern for potential overdosing and chemotherapy associated toxicities [41]. High mobility group A2 (HMGA2) is a protein that can regulate transcription by altering chromatin architecture and facilitate the assembly of multiprotein complexes of transcriptional factors [42]. It is overexpressed in serous OC tumors, but not in normal ovarian epithelial cells [43]. OCs with high expression of HMGA2 and high BMI negatively affected overall survival [44].

Lastly, obesity may increase tumor aggressiveness. Increased metabolic activity and glucose concentrations driven by the Warburg effect [45] are associated with highly aggressive OC cell lines [46]. In cancer, the Warburg effect is regarded as a characteristic metabolic process that may contribute to cell survival in a stressful environment, such as the stress of chronic inflammation [47]. The Warburg effect suggests that cancer cells produce energy predominately by glycolysis and lactic acid production over oxidative phosphorylation [45]. An in vivo obese mouse model demonstrated increased tumor size and tumors in these obese mice had a unique molecular makeup noted by upregulation of inflammation genes [48]. Obesity in OC patients may further exacerbate disease by contributing to an inflammatory environment. Obesity-related type 2 diabetes is associated with chronic inflammation [49-51] and IL-1β levels have been shown to be correlated with obesity and obesity related disorders. Individuals with combined elevated plasma levels of IL-1β and IL-6 are at increased risk for developing type 2 diabetes [52], but even mild weight loss in obese patients resulted in a 45% decrease in serum IL-1β levels over a three-year study period [53]. Leptin, an adipocytokine involved in the pathogenesis of insulin resistance necessary for developing type 2 diabetes, induces β-cell apoptosis and impaired β-cell function by promoting IL-1β production in human pancreatic islets [54]. Expression of leptin is also positively correlated with BMI [55]. In the current study, obese patients tended to have the highest urinary IL-1β levels and increased urinary IL-1β may be indicative of advanced disease. Consequently, urinary IL-1β levels/BMI may prove to be useful prognostic indicator of gynecologic disease.

The greatest limitation of urinary IL-1β as a biomarker for OC is kidney function. Inflammatory mediators, including IL-1β, are typically found elevated in the urine and serum of patients with impaired kidney function [56-58]. Furthermore, elevated levels of IL-1β have been reported in vaginal secretions associated with gynecologic infections; however, Basso, et al. were unable to detect IL-1β in patient urine or serum [59]. Interestingly, while the pH of our urine samples were within neutral range suggesting the absence of urinary tract infections and normal renal function, two samples in our study, 3119–2 and 3119–13, displayed unusually elevated urinary IL-1β levels. Unfortunately, clinical information pertaining to kidney injury/dysfunction or gynecological infection was unavailable as it may have contributed to excessive urinary levels of IL-1β. Clearly, the potential for confounding clinical parameters to influence the impact of urinary IL-1β levels for gynecologic disease warrants further investigation.

The data in this study are derived from a small pilot study, but is representative of our institutional clinical practice in regard to OC histology and stage distribution as well as published pilot studies examining IL-1β in urine, serum, and plasma [17,56,58-61] and other clinically relevant pilot studies [62-66]. A normal baseline value for urinary IL-1β in women has not yet been established in the literature [67]. Therefore, further study with increased sample size may assist in the development of statistically significant baseline and threshold values that could be used to differentiate between healthy, benign disorders, and OC, as well as other clinical parameters such as metabolic disruption. Further, urinary levels of IL-1β levels/BMI as a prognostic indicator of gynecologic disease could impact clinical practice.
Conclusions
Overall, our pilot study suggests that elevated urinary IL-1β may be associated with cancer progression so that the identification of an inflammatory profile specific to epithelial OC may benefit non-invasive diagnostic and prognostic applications as well as lead to the development of adjuvant therapies utilizing target-specific anti-inflammatory treatments to reduce the mortality associated with this disease.

Abbreviations
BMI: Body mass index; EDTA: Ethylenediaminetetraacetic acid; ELISA: Enzyme linked immunosassay; FIGO: International Federation of Gynecology and Obstetrics; HMGA2: High mobility group A2; IL-1β: Interleukin-1β; MCC: H. Lee Moffitt Cancer Center; OC: Ovarian cancer; SD: Standard deviation; SE: Standard error; TVS: Transvaginal ultrasonography; USF: University of South Florida.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
The contributions of each author have been significant: KTW performed the ELISA experiments, analyzed the data, wrote the manuscript and prepared figures for this manuscript. MSH procured clinical specimens, SVN verified histologic diagnoses and MSH and JK collected de-identified clinical information. As PI, PAK developed and oversaw this project from its planning through execution and preparation of the present manuscript. All authors read and approved the final version of the manuscript.

Acknowledgements
This work was supported, in part, by a grant from the Ovarian Cancer Research Fund GRT 10935 (PAK) and a National Sciences Foundation Florida-Georgia Louis Stokes for Alliance Minority Participation (FGLSAMP) Bridge to the Doctorate award HR08 0029435 (KTW).

Received: 26 June 2014 Accepted: 26 October 2014
Published online: 18 November 2014

References
1. American Cancer Society: Cancer Facts & Figures 2013. In Atlanta: American Cancer Society, 2013.
2. Nguyen L, Cardenas-Goicoechea SJ, Gordon P, Curtin C, Momeni M, Chuang L, Fishman D: Biomarkers for early detection of ovarian cancer. Women’s Health (Lond Engl) 2013, 9(2):171–185, quiz 186–177.
3. Cragun JM: Screening for ovarian cancer. Cancer Control 2011, 18(1):16–21.
4. American College of Obstetricians and Gynecologists: PROLOG Gynecology and Surgery. 6th edition. Washington, D.C: American College of Obstetricians and Gynecologists, 2009.
5. Markowska J, Maney G, Kubaszewska M: Value of CA 125 as a marker of ovarian cancer. Eur J Gynaecol Oncol 1992, 13(4):360–365.
6. Carlson KJ, Skates SJ, Singer DE: Screening for ovarian cancer. Ann Intern Med 1994, 121(2):124–132.
7. Su Z, Graybill WS, Zhu Y: Detection and monitoring of ovarian cancer. Clin Chim Acta 2013, 415:341–345.
8. Lewis AM, Varghese S, Xu H, Alexander HR: Interleukin-1 and cancer progression: the emerging role of interleukin-1 receptor antagonist as a novel therapeutic agent in cancer treatment. J Transl Med 2006, 4:48.
9. Mustea A, Pivulescu C, Konsgen D, Braicu EL, Yuan S, Sun P, Lichtenecker W, Sehoul J: Decreased IL-1 RA concentration in ascites is associated with a significant improvement in overall survival in ovarian cancer. Cytokine 2008, 42(1):77–84.
10. World Health Organization: Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser 2000, 894:1–253.
11. Dinarello CA: Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 2009, 27:519–550.
12. Mantovani A, Allavena P, Sica A, Balkwill F: Cancer-related inflammation. Nature 2008, 454(7203):436–444.
13. Kislevsky R, Tolwinski A, Mazurek A, Laudanski P: Inflammation and ovarian cancer—current views. Ginekol Pol 2013, 84(4):293–297.
14. American College of Obstetricians and Gynecologists’ Committee on Gynecologic Practice and The Practice Committee of the American Society for Reproductive Medicine: Female age-related fertility decline. Committee Opinion No. 589, Fertil Steril 2014, 101(3):633–634.
15. Gerard N, Caillaud M, Martoriat A, Gouret G, Lalanchac AC: The interleukin-1 system and female reproduction. J Endocrinol 2004, 180(2):203–212.
16. Caillaud M, Duchamp G, Gerard N: In vivo effect of interleukin-1beta and interleukin-1RAA on oocyte cytoplasmatic maturation, ovulation, and early embryonic development in the mare. Reprod Biol Endocrinol 2005, 3:26.
17. Vural P, Acikol C, Carbaz M: Effects of hormone replacement therapy on plasma pro-inflammatory and anti-inflammatory cytokines and some bone turnover markers in postmenopausal women. Pharmacol Res 2006, 54(4):298–302.
18. Dinarello CA. The paradox of pro-inflammatory cytokines in cancer. Cancer Metastasis Rev 2006, 25(3):307–313.
19. Burney RO, Giudice LC: Pathogenesis and pathophysiology of endometriosis. Fertil Steril 2012, 98(3):511–519.
20. Vassiliadis S, Relakis K, Papageorgiou A, Athanassakis I: Endometriosis and infertility: a multi-cytokine imbalance versus ovulation, fertilization and early embryo development. Clin Dev Immunol 2005, 12:125–129.
21. Dobryczka B, Mackowiak-Matejeczyk B, Terlikowska KM, Kulesza-Brzyczk B, Kinalski M, Terlikowski SJ: Serum levels of IL-6, IL-8 and CRP as prognostic factors in epithelial ovarian cancer. Eur Cytokine Netw 2013, 24(5):106–113.
22. Iodama J, Miyagi Y, Seki N, Tokumo K, Yoshinouchi M, Kobayashi Y, Okuda H, Kudo T: Serum C-reactive protein as a prognostic factor in patients with epithelial ovarian cancer. J Obstet Gynecol Reprod Biol 1999, 82(1):107–110.
23. Heffer-Frischmuth K, Heffer LA, Heinze G, Paseka V, Grimm C, Tempfer CB: Serum C-reactive protein in the differential diagnosis of ovarian masses. Eur J Obstet Gynecol Reprod Biol 2009, 147(1):65–68.
24. Brownie A, Strakas R, Guney T, Rama N, Van Noorden S, Curry E, Gabra H, Stornach E, ElBahrawy M: Differential expression of IL-8 and IL-8 receptors in benign, borderline and malignant ovarian epithelial tumours. Cytokine 2013, 64(1):413–421.
25. Lim D, Oliva E: Precursors and pathogenesis of ovarian carcinoma. Pathology 2013, 45(3):229–242.
26. Collaborative Group on Epidemiological Studies of Ovarian Cancer: Ovarian cancer and body size: individual participant meta-analysis including 25,157 women with ovarian cancer from 47 epidemiological studies. PLoS Med 2012, 9(4):e1001200.
27. Olsen CM, Nagle CM, Whitman DC, Ness R, Pearce CL, Pike MC, Rossing MA, Terry KL, Wu AH, The Australian Cancer Study (Ovarian Cancer), Henderson KD, Ursin G, Horn-Ross PL: Interleukin-1 and cancer by hormone therapy use in the California teachers study cohort. Cancer Epidemiol Prev 2009, 25(157):1–22.
28. Leitzmann MF, Koebnick C, Danforth KN, Brinton LA, Moore SC, Hollenbeck AR, Schatzkin A, Lacey Jr N: Body mass index and risk of ovarian cancer. Cancer 2009, 115(4):812–822.
29. Canchola AJ, Chang ET, Bernstein L, Largent JA, Reynolds P, Deapen D, Henderson KD, Ursin G, Horn-Ross PL: Body size and the risk of ovarian cancer by hormone therapy use in the California teachers study cohort. Cancer Causes Control 2010, 21(12):2241–2248.
30. Delort L, Kwiatkowski F, Chalabi N, Satih S, Bignon YJ, Bernard-Gallon DJ: Central adiposity as a major risk factor of ovarian cancer. Endocr Relat Cancer 2010, 20(1):251–262.
31. Leitzmann MF, Schatzkin A, Lacey Jr N: Body mass index and risk of ovarian cancer. Cancer 2009, 115(4):812–822.
32. Canchola AJ, Chang ET, Bernstein L, Largent JA, Reynolds P, Deapen D, Henderson KD, Ursin G, Horn-Ross PL: Body size and the risk of ovarian cancer by hormone therapy use in the California teachers study cohort. Cancer Causes Control 2010, 21(12):2241–2248.
33. Delort L, Kwiatkowski F, Chalabi N, Satih S, Bignon YJ, Bernard-Gallon DJ: Central adiposity as a major risk factor of ovarian cancer. Endocr Relat Cancer 2009, 20(12):5229–5234.
34. Schouten LJ, Rivera C, Chang-Claude J, Hein R, Nickels S, Wang-Gohrke S, Goodman MT, Carney ME, Matsuno R, Lurie G, Moysich K, Kjaer A, Hodgall E, Goode E, Frielied BL, Verkiant RA, Larson MC, Schildkraut J, et al Obesity and risk of ovarian cancer subtypes: evidence from the ovarian cancer association consortium. Endocr Relat Cancer 2010, 20(1):251–262.
35. Leitzmann MF, Koebnick C, Danforth KN, Brinton LA, Moore SC, Hollenbeck AR, Schatzkin A, Lacey Jr N: Body mass index and risk of ovarian cancer. Cancer 2009, 115(4):812–822.
Hotamisligil GS: Inflammation and metabolic disorders. Nature 2006, 444(7121):860–867.

Spranger J, Kroke A, Mohlig M, Hoffmann K, Bergmann MM, Ristow M, Boering H, Pfeiffer AF: Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. Diabetes 2003, 52(3):812–817.

Chae JS, Paik JK, Kang R, Kim M, Chai Y, Lee SH, Lee JH: Mild weight loss reduces inflammatory cytokines, leukocyte count, and oxidative stress in overweight and moderately obese patients treated for 3 years with dietary modification. Nutr Res 2013, 33(3):195–203.

Maedler K, Sergeev P, Ehes JA, Matze V, Bosco D, Berney T,ayer JM, Reinecke M, Halban PA, Donath MY: Leptin modulates beta cell expression of IL-1 receptor antagonist and release of IL-1beta in human islets. Proc Natl Acad Sci U S A 2004, 101(21):8138–8143.

Diaz ES, Karlan BY, Li AJ: Obesity-associated adipokines correlate with survival in epithelial ovarian cancer. Gynecol Oncol 2013, 129(2):353–357.

Hong MY, Tseng CC, Chuang CC, Chen CL, Lin SH, Lin CF: Urinary macrophage migration inhibitory factor serves as a potential biomarker for acute kidney injury in patients with acute pyelonephritis. Meditators inflam. 2012, 2012:31938.

Pruim M, Ponte B, Vollenweider P, Moeser V, Passaud F, Waeber G, Vichare V, Marques-Vidal P, Burnier M, Bochud M: Not all inflammatory markers are linked to kidney function: results from a population-based study. Am J Nephrol 2012, 35(3):288–294.

Mozseyenok VM, Chubenyak VA, Mozseyenok FV, Zhabina AS, Gorodnova TV, Komarov YI, Bogdanov AA, Sokolenko AP, Imyanitov EN: Evidence for clinical efficacy of mitomycin C in heavily pretreated ovarian cancer patients carrying germ-line BRCA1 mutation. Med Oncol 2014, 31:139.

Hjerppe E, Brage SE, Frostvik SM, Johannson H, Shoshan M, Avall-Lundqvist E: Macrophage markers and HSP60 in chemonefa, solid ovarian cancer versus ascites. Int J Gynecol Cancer 2014, 24(5):1389–1394.

Gabrielsson M, Bjerklund C, Carlson J, Shoshan M: Expression of mitochondrial regulators PGC1a and TFAM as putative markers of subtype and chemoresistance in epithelial ovarian carcinoma. PLoS One 2014, 9(9):e107109.

Bogush TA, Didko EA, Semakova AV, Bogush EA, Tjulandin A, Zarkovta V, Tjulandin SA, Davydov MI: Immunofluorecent assay of ERCC1 and estimation of clonal significance of the protein expression in ovarian cancer tissue. Biochem Biophys Mol Biol 2014, 457:1410–1445.

Iakimovska M, Ceron K, Verdenik J, Kobal B: Circulating serum sVCAM-1 concentration in advanced ovarian cancer patients: correlation with concentration in ascites. Radial Oncol 2014, 4(3):307–313.

Bourgeois MM, Richards IS: Gender-specific differences in the urinary expression of aldosterone, IL-1alpha and IL-1beta. Biomark Med 2010, 4(3):843–847.