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Autoantibodies as biomarkers for ovarian cancer

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Abstract

Ovarian cancer (OVCA) has the highest mortality of all gynecologic cancers. The poor survival rate is due to the lack of diagnostic screening tests and high incidence of recurrence in OVCA patients resistant to chemotherapy that leads to a more aggressive form of the disease. Therefore, a search for biomarkers holds great promise not only for early detection of OVCA at presymptomatic stage and for monitoring the course of the disease during the first-line chemotherapy treatment but also for identifying those women whose disease is likely to recur. Research efforts have sought to unravel the complexity of the tumor specific proteome by profiling immune responses generated against tumor associated antigens (TAAs) using multianalyte-based analytical discovery platforms readily adaptable to clinical diagnostic screening tests. The occurrence of tumor-specific autoantibodies directed to respective TAAs can be observed before the development of clinical symptoms. Evaluation of the level of tumor autoantibodies during the time of tumor debulking followed by first-line chemotherapy for the prediction of early recurrence as well as their correlation with other clinical parameters to evaluate their prognostic value has been conducted in various clinical studies. The anti-tumor immune response against OVCA is the ultimate key to the development of multiple immune-based therapeutic strategies that have been proposed and tested in different clinical trials that may have beneficial impact on the disease outcome in OVCA patients.

Keywords

Ovarian cancer; humoral immune response; tumor autoantibodies; early detection; recurrence; immunotherapy

1. Introduction

Ovarian cancer (OVCA) is the fifth most common cause of cancer death in women, with more than 21,880 new cases reported in the US in 2010.[1] Current diagnostic tools for screening ovarian cancer include measurement of the level of serum tumor markers using a blood tests and/or ultrasound imaging of the ovaries. A variety of ovarian tumor markers have been studied and the most extensively investigated of these is CA125. Despite the applicability of CA125 in a clinical setting for monitoring recurrence of disease, this test has a very low sensitivity for detecting OVCA at an early stage because CA125 is elevated in only about 50% of patients with clinically detectable early stage OVCA [47]. However,

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[1]http://caonline.amcancersoc.org/cgi/reprint/60/5/277?ijkey=c47 c66bfb1f12623e0568c66974f0ab927333ce6.

multimodal screening for OVCA using CA125 with various other tumor markers effectively increases the sensitivity for early detection. Zhang et al. reported that by using CA125II, CA72-4, CA15-3, and macrophage colony stimulating factor (M-CSF) as inputs to an artificial neural network (ANN) derived using a training set comprising of healthy women (n = 100), benign conditions (n = 45), invasive epithelial ovarian cancer (EOC) (n = 55). An independent test set comprising of healthy women (n = 98), early stage EOC (n = 52; 38 stage I, 4 stage II invasive cases, and 10 stage I borderline ovarian tumor cases) was used for evaluation of ANN. ROC analyses revealed that at a fixed specificity of 98%, the sensitivities for ANN and CA125II alone were 71% (37/52) and 46% (24/52) (p = 0.047) for the detection of early stage epithelial ovarian cancer (EOC), and 71% (30/42) and 43% (18/42) (p = 0.040) for the detection of invasive early stage EOC [79]. Yurkovetsky et al. reported that four biomarkers panel comprised of CA125, HE4, CEA, and VCAM1 was able to detect early stage OVCA with 86% sensitivity and 98% specificity using multiplex xMAP bead-based immunoassays. In that same population, CA125 alone had a sensitivity of 61% for early stage OVCA [77].

Most of these serum tumor antigens are released from tumor cells by secretion or shedding [44,62], then enter into circulation and eventually are captured by antigen processing machinery for proper antigen presentation. This antigenic presentation of tumor peptide epitopes in conjunction with MHC class II molecules can result in humoral immune response in cancer patients resulting in the formation of a huge repertoire of tumor reactive immunoglobulins [68]. The detection of serum antibody responses to tumor antigens may provide more reliable serum biomarkers for cancer diagnosis because serum antibodies are more stable compared to serum antigens. Circulating serum antigens are labile and have shorter half lives. For example, the reported half lives of CEA, CA19-9 and AFP were 1.5 days, 0.5 days and 1 day in patients after removal of intrathoracic malignancies [76], and the half life of S100B protein in melanoma patients was reported to be 30 min [25]. In contrast, antibodies are more abundant than antigens, especially at low tumor burdens of early stage of cancers and their role as reporters of early or incipient carcinogenesis has been well documented. Abendstein et al. reported that anti-p53 antibodies may develop months to years before the clinical diagnosis of cancer [1]. Numerous intracellular proteins can elicit humoral immune responses in different cancer patients [68] as a result of aberrant expression of antigen biomarkers [9], alternative splicing of pre-messenger RNAs [73], point mutations [74] and overexpression [15], or post-translation alterations of the expressed antigens such as changes in glycosylation [72], phosphorylation [16]. Thus, different changes that occur in the structure or expression pattern of certain cellular components during tumorigenesis can trigger the immune system to recognize antigens as non-self. Although T-cells encounter most of the self antigens due to promiscuous gene expression by medullary thymic epithelial cells [38] during their maturation in thymus and get tolerized, growing evidence still supports the presence of self-reactive T-cells in the T-cell repertoire. Studies have shown that only properly processed self-antigen determinants are able to tolerize T-cells. However, self-antigens may have ‘subdominant’ or ‘cryptic’ determinants that are poorly processed from native molecule and inefficiently presented to T cells [11]. When these self-antigens are overexpressed in cancer, the cryptic determinants are eventually presented to T-cells in a co-stimulatory environment thus eliciting immunological responses [52]. Although occurrence of tumor autoantibodies as a result of activation of immune responses towards various TAAs in cancer patients could be extremely beneficial in vaccine development, reports from different studies indicate that repertoire of tumor autoantibodies overlaps to a significant extent with the typical patients with autoimmune diseases. Therefore, a panel of good candidate TAAs for cancer immunotherapy should be selected in such as way that activation of immune responses against those TAAs will have favorable clinical outcomes without the risk of autoimmunity. This review will present an overview of tumor autoantibodies in OVCA as biomarkers for i) early diagnosis, ii)

prediction of prognosis, iii) prediction of recurrence and iv) developing therapeutic approaches such as immunotherapy for management of OVCA.

2. Tumor antibodies in OVCA

2.1. Usefulness of tumor autoantibodies for early diagnosis of OVCA

With an ovarian cancer prevalence of 1 in 2500 among postmenopausal women in the United States, an effective screening strategy for the general population needs to attain a sensitivity of 75% and specificity of 99.7% to attain a minimally acceptable positive predictive value of 10% for the detection of OVCA [31,49]. Approximately 75% of OVCA patients are diagnosed in late stage disease stage (III–IV) and have a 5-year survival rate of only 15–20%, compared to a 80–90% survival rate when the cancer is detected at early stage (I–II) [65]. Autoantibodies to TAAs develop at very early stage, well before the clinical manifestation of the disease because of the triggering of humoral immune responses due to the presence of otherwise undetectable amounts of TAAs at very low tumor burdens. Thus, antibodies against tumor specific proteins potentially provide candidate early biomarkers for detecting ovarian cancer at a curable stage (Table 1).

In human carcinogenesis, p53 gene exhibits genetic alterations such as missense point mutations that can lead to a conformational change thereby increasing the stability of p53 protein allowing it to accumulate in the nucleus [24,67]. This relative increase in the amount of mutant p53 protein, acting as an immunogen, can trigger humoral immune response leading to the generation of anti-p53 antibodies in tumor microenvironment [19]. In 1982, Crawford et al. first described antibodies against p53 protein in 9% of breast cancer patient sera and the presence of p53 antibodies indicated that immunogenicity of p53 protein could be associated with its altered amount or different presentation in breast tumors [13]. Circulating antibodies to p53 protein in ovarian cancer have been previously reported by Gadducci et al. [19]. Using enzyme-linked immunosorbent assay (ELISA), this group reported that preoperative serum anti-p53 antibodies were found in 3 (10.0%) of the 30 patients with stage I–II and 15 (26.8%) of the 56 patients with stage III–IV epithelial ovarian cancer ($P = 0.09$). Their study indicated that sero-positive patients had higher titers of anti-p53 antibodies when their tumors showed p53 overexpression. Anti-p53 antibodies were not found in late stage (III–IV) patients who had well differentiated tumors. However, p53 autoantibodies were observed in 30.6% patients who had moderately and poorly differentiated tumors. Their study concluded that incidence of p53 antibodies was higher is advanced ovarian tumor stage and grade although the differences between poor and well-differentiated tumors were not statistically significant. Vogl et al. conducted a hospital-based cohort study comprising of 113 patients with ovarian cancer, 15 patients with borderline tumors and 117 patients with benign tumors of the ovaries. With the use of newly developed ELISA based on highly purified and re-natured p53, the prevalence of autoantibodies for p53 protein in patients with invasive cancer was found to be 19% (21/113), whereas, no p53 antibodies were observed in patients with ovarian borderline or benign tumors. These findings indicated that p53 antibodies were highly specific for malignancy in patients with ovarian mass and correlated well with aggressive ovarian cancer [69].

Using a high-throughput cloning method in combination with serological profiling of immunoreactive antigens on protein microarrays called Epitomics, Chatterjee et al. reported the identification of 65 antigens that discriminated OVCA patients from normal healthy women or women with other benign/gynecological diseases [8]. Immunoscreening of antigen macroarrays using samples from discovery set comprising of 32 ovarian cancer patients, 25 healthy women and 14 patients having other benign or malignant gynecological diseases revealed that the reactivity of 65/480 antigens. These 65 antigen biomarkers were further validated on a larger set of 129 independent samples comprised of stage I invasive

OVCA ($n = 20$), borderline OVCA ($n = 3$), late stage OVCA ($n = 46$), healthy female controls ($n = 60$) using protein microarrays. Neural network analyses with these 65 biomarkers revealed an average sensitivity and specificity of 55% and 98% respectively. This study indicated that a panel of 65 antigens could provide useful diagnostic markers for the early detection of OVCA especially in high-risk populations. The cause of immunogenicity of 4 of these previously identified 65 TAAs was later confirmed in a follow-up study which demonstrated that the occurrence of humoral immune responses against some of these TAAs in OVCA patients is triggered by antigen protein overexpression [2].

In another study Li et al. reported the diagnostic potential of panel of autoantibodies against TAAs for the detection of OVCA [40]. Their pilot study population was comprised of 32 OVCA patients and 82 normal individuals. Using an ELISA method that was later confirmed by immunoblotting analysis, this group found that the sensitivity and specificity of the panel of 13 TAAs was 62.5% and 85.4% respectively. They also reported that with the addition of 7 more known TAAs, there was a stepwise increase in sensitivity of up to 62.5% and in specificity of 90.2%.

Naora et al. by immunoscreening a cDNA expression library with ovarian cancer patient serum using SEREX technology identified HOXB7 protein a homeobox gene product,elicited a humoral immune response in ovarian carcinoma patients. ELISA assay using purified recombinant HOXB7 protein revealed significant serologic reactivity to HOXB7 in 13 of 39 ovarian cancer patients and in only one of 29 healthy women ($P < 0.0001$). Their study showed that serological detection of autologous antibodies to HOXB7 could have diagnostic potential for detection of ovarian cancer [53]. Follow-up studies need to be performed to validate the diagnostic utility of HOXB7 autoantibodies in a larger population-based case-control study.

The prevalence of tumor-specific antibodies to heat shock protein-90 (HSP-90) were found to be highest in the sera of late stage OVCA. In the study performed by Luo et al., recombinant HSP-90 protein was used to detect level of autoantibodies in sera obtained from 10 OVCA stage (I–II), 22 OVCA stage (III–IV), 37 colorectal cancer, 13 breast cancer, 10 lung cancer, 20 benign gynecologic disease, 10 benign breast lesions, and 20 normal females using ELISA. Using fluorescence ratio cutoff was 2.00 (mean +2 standard deviations) 1 (10%) stage (I–II) OVCA, 7 (32%) stage (III–IV) OVCA, 1(3%) colorectal cancer, 1(8%) breast cancer, and 1(5%) benign/gynecological disease were found to have elevated levels of HSP-90 antibodies. Even when the specificity was set to 100% (fluorescence ratio > 2.3), 6 late stage OVCA, 1 early stage OVCA, 1 colorectal and 1 breast cancer patient showed reactivity to HSP-90. The authors concluded that HSP-90 autoantibodies were mostly present in late stage OV-CA and it might be a biomarker for epithelial ovarian carcinoma [45]. Kim et al. reported the presence of autoantibodies against a novel protein called stress-induced phosphoprotein-1 (STIP-1) by applying two-dimensional differential gel electrophoresis analysis of immuno-precipitated tumor antigens (2D-DITA) in OVCA patients. Using an ELISA method, this group evaluated the level of STIP-1 antibodies in the plasma samples obtained from 63 OVCA patients, 13 borderline ovarian tumors, and 63 healthy individuals. The difference in the level of autoantibodies against STIP-1 between ovarian cancer and healthy controls was statistically significant ($P = 0.03$). Among the cancer patients, serous histology OVCA showed significantly higher levels of STIP-1 antibodies compared to other histological types of OVCA ($P = 0.001$) [35]. Their results indicated that STIP-1 autoantibodies might be a potential biomarker candidate for OVCA.

Cancer–testis (CT) antigens belong to a category of tumor antigens that show restricted expression only in male germ cells (testis). CT antigens are overexpressed in a significant subset of malignant tumors but they are not expressed in adult somatic tissues [63]. These

antigens are highly immunogenic and have shown to elicit humoral immune response in cancer patients [64]. Odunsi et al. reported humoral immune response to recombinant CT antigens such as NY-ESO-1 and LAGE-1 proteins in OVCA patients by applying ELISA methods [54]. LAGE-1 gene bears 94% homology to NY-ESO-1 gene and 180 amino acid proteins encoded by the fully spliced LAGE-1 mRNA and NY-ESO-1 mR-NA display 84% identity [39]. Their results showed that NY-ESO-1/LAGE-1 antibodies were present in 11/37 (30%) of patients (mostly stage IIIC, papillary serous histology) with tumors showing expression of NY-ESO-1 or LAGE-1 antigens. Only one patient who tumor showed no expression of NY-ESO-1 and LAGE-1 showed sero reactivity to NY-ESO-1. Detectable autoantibodies were present in sera of OVCA patients for up to 3 years after initial diagnosis. Their data showed aberrant expression of NY-ESO-1 and LAGE-1 antigens in significant proportion of epithelial OV-CA patients. Thus, antibodies to NY-ESO-1/LAGE-1 might serve as a candidate biomarker of epithelial ovarian carcinoma. Another novel CT antigen called testis-specific sperm-associated antigen 9 (SPAG9) has been reported to elicit humoral immune response in OVCA. Using ELISA and western blot methods Garg et al. reported the immunoreactivity of SPAG9 antigen in 11/19 (58%) serous adenocarcinoma, in 2/2 (100%) clear cell carcinoma, and in 3/3 (100%) mucinous Adenocarcinoma [22]. This group also noted that 5/8 (62.5%) of patients suffering from various histotypes with early stages (I–II) of OVCA elicited humoral immune response to SPAG9. Similarly, 15/22 (68%) of late stage (III–IV) OVCA patients revealed immunogenicity against SPAG9 antigen *in vivo*. These findings indicated that antibodies against SPAG9 might be a candidate biomarker of early diagnosis of OVCA.

Using a new immunofluorescent bead-based Luminex technology, Lokshin et al. [42] reported the detection of IL-8 autoantibodies in immunoassays of serum samples obtained from 44 patients with early stage (I–II) OVCA, 50 patients with late stage (III–IV) OVCA, 37 patients with benign pelvic masses, and 80 healthy women. Their study indicated that concentrations of both IL-8 cytokine and IL-8 antibodies were elevated in the sera of ovarian cancer patients when compared to healthy individuals. Logistic regression analysis of the concentrations of circulating autoantibodies to IL-8 was able to discriminate patients with early stage (I–II) OVCA from healthy controls with 98% specificity, 65.5% sensitivity. A combination of anti-IL-8 antibody and CA125 assays resulted in increased diagnostic potential of the test that suggested the circulating IL-8 antibodies might serve as potential diagnostic marker for OVCA.

Humoral immune responses to MUC1 protein, a transmembrane *O*-linked glycoprotein present on the apical surface of normal secretory epithelial cells have been reported in many cancers [60] and the presence of immune-complexed MUC1 in cancer patients is related to a favorable disease outcome [71]. Using ELISA assay applying 100-mer synthetic MUC1 peptide (corresponding to five tandem core repeats of MUC1 polypeptide), Cramer et al. determined the level of MUC1 antibodies in the plasma samples obtained from 668 patients (with EOC; histology subtype: serous borderline, serous invasive, mucinous, endometroid and other/undifferentiated) and 721 control individuals [12]. Their data revealed that a cutoff of optical density A >= 0.6, 33.8% of controls and 45.8% of cases were positive for antibodies to MUC1. By using a cutoff of A >= 1.0, 12.3% of control individuals and 25% of OVCA patients were shown to have a high level of antibodies to MUC1 and that this significant difference in the level of MUC1 antibodies between OVCA patients and controls might reflect the presence of an ongoing immune response to tumor in OVCA patients necessary for its detection at early stage.

S100A7 (psoriasin), a member of S100 EF-hands calcium-binding signaling protein,which plays a role in inflammation processes, has been shown to trigger humoral immune response in OVCA. Gagnon et al. identified S100A7 by applying 2D–DITA analysis of

immunoprecipitated tumor antigens and using an ELISA assay this group quantified the autoantibodies against S100A7 protein in the plasma samples obtained from 23 early-stage OVCA, 69 late-stage OVCA, 11 benign gynecologic disease, and 35 age-matched healthy individuals. By applying nonparametric Mann-Whiteney U test, a significant difference between the levels of autoantibodies to S100A7 was observed in early and late stage OVCA when compared to healthy controls. ($P = 0.05$ and $P < 0.001$ respectively). A significant difference was not observed between healthy controls and patients with other benign gynecologic disease ($P = 0.07$). The author suggested based on overexpression of S100A7 protein in all histologic subtypes of OVCA [20] and the generation of humoral immune responses in OVCA patients, that the autoantibodies against S100A7 alone or in combination with other tumor autoantibodies can be used as diagnostic markers for OVCA screening.

Thus, there is a collateral benefit of the surrogate role of these autoantibodies as diagnostic biomarkers in that they identify TAAs that are involved in pathogenesis of ovarian cancer may prove useful in evaluating survival rates in cancer patients as well as provide earlier diagnosis.

2.2. Usefulness of tumor autoantibodies for prediction of OVCA prognosis

The management of advanced OVCA remains a significant challenge because of tumor heterogeneity and many patients fail to respond to therapy,resulting in disease progression leading to increased morbidity rates among OVCA patients. A biomarker that could rapidly predict the disease outcome would be extremely beneficial in allowing the administration of patienttailored therapy while reducing toxicity and cost. Coupling tumor biomarkers to clinicopathologic tumor variables such as tumor size, grade, and stage may be ideal combination to predict disease outcome. Growing evidence suggests that OVCA patients can mount humoral immune response against TAAs thus generating a large repertoire of tumor autoantibodies that may impart a survival benefit. The existent humoral immunity can be augmented further by different clinical strategies (see immunotherapy section) that will be used for immunotherapy of OVCA.

The prognostic value of anti-p53 antibodies in serous OVCA was evaluated by Anderson et al. In order to assess the association between the presence of p53 autoantibodies and survival, they reviewed the medical records of 60 serous cases (grade 3 and 95% stage (III–IV). The clinical and treatment characteristics of all the serous patients were obtained for the survival analysis. Fifty nine out of 60 patients received adjuvant chemotherapy. The statistical model, adjusting for age, year of diagnosis, platinum containing chemotherapy and number of cycles of chemotherapy, stage and laboratory batch number, showed that anti-p53 antibodies in serous OVCA patients were associated with improved survival (hazard ratio = 0.57; 95% confidence interval (95% CI), 0.33–0.97; $P = 0.04$) [3]. In 2006, Goodell et al. assessed the presence of tumor specific antibodies to p53 in 104 late stage (III–IV) OVCA in order to predict overall survival in advanced stage OVCA patients. Statistical analyses showed that patients who had antibodies to p53 had significantly higher survival than patients without 53 antibodies ($P = 0.01$). The median survival of patients positive for p53 antibodies was 51 months (95% CI, 23.5 to 60.5 months) compared with 24 months (95% CI, 19.4 to 28.6 months) for patients who did not have p53 autoantibodies. Their results indicated that p53 autoantibodies may predict improved overall survival in patients with advanced stage OVCA [23]. In contrast, in a different study population comprised of 44 OVCA patients stage (III– IV), who underwent first-line treatment with 6 cycles of chemotherapy, Gadducci et al. reported that at the end of the treatment, a pathological complete response at second-look was achieved by none of the patients with anti-p53 antibodies compared to 24.1% of patients without p53 antibodies ($P = 0.09$). The

authors concluded that serum anti-p53 antibodies had no prognostic relevance for progression-free survival in patients with advanced stage OVCA [19].

MUC1 autoantibodies were shown to have prognostic value in predicting survival in OVCA patients by Richards et al. [57]. This group utilized a synthetic peptide (105 amino acid segment) bearing epitopes on the core protein of MUC1 and measured the MUC1 antibodies in the serum of healthy pregnant and nonpregnant women, and in patients with benign and malignant ovarian tumors. It was found that MUC1-reactive antibodies were IgM isotype and regardless of age, the level MUC1 antibodies in cancer patients was much lower than in healthy women ($P < 0.0001$). Although univariate analysis revealed association of high antibody levels with greater survival in OVCA ($P = 0.015$), multivariate regression analysis showed that after consideration of stage, histological subtype, serum MUC1 levels and age, autoantibodies to MUC1 failed to act as a significant independent prognostic indicator. Cramer et al. in another study reported that MUC1 antibodies were shown to be inversely associated with ovarian cancer risk. Their study showed that compared to women who experienced oral contraceptives, tubal sterilization, mastitis, and bone fracture,or women who experienced more of these conditions had higher levels of antibodies to MUC1. In parallel, the adjusted ovarian cancer risk decreased progressively with relative risks (and 95% confidence limits) of 0.69 (0.52–0.92), 0.64 (0.47–0.88), 0.49 (0.34–0.72), and 0.31 (0.16–0.61), respectively for women with two, three, four, and five or more conditions related to the presence of MUC1 antibodies ($P_{trend} < 0.0001$) [12].

Mesothelin is overexpressed in a variety of cancers including mesothelioma, OVCA and pancreatic cancer [26]. Mesothelin has a CA125 binding domain and the interaction between the two may facilitate the implantation and spread of tumor in the peritoneal cavity by cell adhesion [32]. The significance of mesothelin expression with clinical outcome in OVCA was reported by Yen et al. In their study, mesothelin expression was evaluated by immunochemistry in tumor specimens obtained from 198 serous ovarian carcinoma patients. This study showed that mesothelin immunoreactivity was present 55% of serous ovarian carcinomas with similar expression in both high and low grade tumors ($P = 0.82$). Among 105 high grade cases, who received optimal debulking followed by chemotherapy, patients whose tumor specimens showed diffuse immunoreactivity (immunostaining score: 2–4) had significantly better overall survival than patients with tumors showing negligible or focal immunoreactivity (immunostaining score: 0–1) ($P = 0.023$, log-rank test) [75]. In 2005, Ho et al. reported a study based on the determination of immunogenicity of mesothelin by profiling humoral immune response to mesothelin in OVCA patients. Their results showed that mesothelin specific antibodies were elevated in 41.7% (10/24) of epithelial OVCA patients when compared with normal population ($n = 44$; $P < 0.01$). About 56% of patients with mesothelin immunostaining positive OVCA also had anti-mesothelin antibodies, whereas 0% to 8% of patients with negative mesothelin immunostaining had detectable mesothelin specific antibodies (χ^2 test: $P = 0.025$) [28]. The authors concluded that immunogenicity of mesothelin was associated with its high expression in the tumor cells. Based on the above two studies it may be reasonable to conclude that as higher expression of mesothelin in OVCA is associated with better overall survival [10], it might be enlightening to evaluate the level of autoantibodies to mesothelin over the course of the patient's disease while the patient is undergoing first-line chemotherapy and to determine if under any clinical situation the mesothelin autoantibodies have anti-tumor protective response. The evaluation of such protective antibody response (if any) to mesothelin may have prognostic and immunotherapeutic values for predicting patient's outcome and cure for OVCA.

Thus, a correlative study between the level of tumor autoantibodies and the overall survival outcome of cancer patients (reflected in the change in tumor status or tumor burden related

to the therapy) (Table 1) is of utmost importance and may permit stratification of patients into meaningful prognostic categories that will be extremely informative for evaluating second-line therapeutic treatments following primary debulking surgery and platinum based first-line chemotherapy.

2.3. Usefulness of tumor autoantibodies for prediction of OVCA recurrence

The current standard of treatment for patients with invasive ovarian cancer is cytoreductive surgery followed by platinum-based chemotherapy. Despite the performance of optimal primary treatment for patients with advanced epithelial OVCA, 60% of these patients will develop abdominal relapse [21]. Although 20% of these OVCA patients have little or no expression of CA125 making it less acceptable as a screening marker, CA125 has provided a used biomarker for monitoring of OVCA and its recurrence [50]. A commentary report from Bast et al. indicated that a large proportion of women (> 50%) with advanced stage disease who are treated with cytoreductive surgery and chemotherapy will respond to therapy with normalization of serum CA125 levels followed by complete clinical remission. If a second-look surgeries are performed, more that 50% women will show the presence of microscopic or macroscopic diseases that usually fall below the threshold limit of detection either by imaging or CA125 level. It has been also reported that even with negative results after second-look surgery, the majority of patients will experience OVCA recurrence within months to years and in about 80% of women an increase in the level of CA125 precedes OVCA recurrence by 3 to 5 months [61]. Report from Prat et al. indicated that an absolute increase of CA125 of 5U/ml over baseline was significant in predicting recurrence of advanced EOC with a median lead time of 58 days in recurrence group compared to non-recurrence group in similar patient cohorts [56]. In another study by Kang et al., it was shown that the optimal cutoff point of CA125 after completing adjuvant chemotherapy to predict disease progression was 12U/ml (sensitivity, 71.4%; specificity, 82.1%). The risk of recurrence was higher for CA125 values > 12 U/ml (hazard ratio = 10.567; $P < 0.001$) [33].

The level of autoantibodies against TAAs has been reported to be informative in predicting recurrence of cancer. Cui et al. reported that autoantibodies against paraneoplastic antigen Ma2 could serve as potential biomarker for detecting recurrence after radical operation of small neuroendocrine tumors [14]. By applying indirect ELISA method, they showed 4/19 patients who had Ma2 antibody below the cutoff level had tumor recurrence during their follow-up. In a different group, 13/17 patients with Ma2 antibody above the cutoff level showed tumor recurrence. These results indicated that Ma2 antibodies can serve as potential biomarker for determining recurrence in small neuroendocrinecancer patients after the radical surgery for these tumors. Kim et al. evaluated the efficacy of anti-thyroglobulin autoantibody (TgAb) testing in predicting recurrence in differentiated thyroid carcinoma (DTC) patients at 6– 12 months after high dose ^{131} I remnant ablation [36]. Their study showed that among 56 patients who had positive TgAb antibodies at 6–12 months, 10 patients (18%) were reported to have recurrence during the median 73.4 months of follow-up, whereas in 10/741 (1%) in the TgAb negative group had recurrence ($P < 0.001$).

To date, very little data on the evidence of tumor autoantibodies in predicting recurrence ovarian cancer patients are available. Reports from Vogl et al. revealed 46% prevalence of circulating p53 autoantibodies in a study population comprising of 83 OVCA patients using ELISA. Their study also indicated that in a bivariate analysis, patients with anti-p53 autoantibodies had a 1.96-fold risk for relapse (95% confidence interval 1.02–3.78) [70].

Ep-CAM (epithelial cell adhesion molecule), known to be involved in cell adhesion processes, signal transduction and gene regulation [41] elicits a humoral immune response in OVCA [34]. Using ELISA, the autoantibody level of Ep-CAM was measured in the sera obtained from 52 OVCA patients, 26 patients with benign ovarian disease and 26 normal

individuals. The data analyses showed that the difference between cancer and non-cancer groups was statistically significant ($P < 0.05$). Based on the cutoff OD value (mean of normals +2 standard deviations), the immunoreactivity against Ep-CAM was found in 22 OVCA patients (42.3%), none of controls (0%) and in 2 benign ovarian disease (7.7%). Using an OD cutoff value of 0.115, obtained by receiver operating curve (ROC) au-toantibodies to Ep-CAM were able to detect epithelial OVCA with 73.1% sensitivity and 80.8% specificity. In a different study conducted by Bellone et al., immunohistochemical analysis of 168 fresh frozen biopsies and paraffin-embedded tissues obtained from epithelial ovarian carcinoma (EOC) patients indicated that Ep-CAM protein expression was significantly higher level in primary, metastatic and recurrent epithelial ovarian carcinoma as compared to normal ovarian tissues. Particularly interesting was that the metastat-ic/recurrent tumors were found to express significantly higher level of Ep-CAM protein compared to primary ovarian carcinoma ($P < 0.001$) [4]. Based on the above two studies overexpression of Ep-CAM in recurrent EOC may trigger humoral immune responses in recurrent EOC patients because Ep-CAM has been previously shown to elicit humoral immune responses in OVCA patients and anti-Ep-CAM autoantibodies have been shown to act as candidate biomarkers for diagnosis of OVCA [34]. Whether anti Ep-CAM antibodies have potential as candidate biomarker for predicting recurrence and monitoring OVCA remains to be seen.

The management of recurrent ovarian cancer is a major clinical hurdle because relapse during or immediately after platinum based first-line chemotherapy results into more aggressive form of platinum-refractory ovarian cancer unlikely to experience an objective response to currently available chemotherapeutic agents. Previous studies have indicated that autoantibodies to TAAs may act as potential candidate biomarkers to predict recurrence in OVCA. Thus, evaluation of the level of tumor autoantibodiesfor monitoring of OVCA while the patient is on first-line treatment will be extremely beneficial for the clinicians to make second-line treatment decision for better prognosis.

2.4. Use of tumor autoantibodies for immunotherapy of OVCA

Growing evidence supports the existence of immunosurveillance mechanisms in OVCA patients. The tumor infiltrating lymphocytes (TILs) are the key immune effector cells that play an important role in immunosurveillance and their presence in the tumor microenvironment has been shown to correlate positively and strongly with patients survival [78]. However, tumors often use various different mechanisms to evade immune responses such as infiltration of T-regulatory cells (Tregs) [66], down-regulation of the antigen-processing machinery such as MHC class I molecules has been observed in different cancers like breast, prostate and lung cancer [48]. Down-regulation of the transporter associated with antigen presentation (TAP) genes as well as components of the immunopro-teosome such as LMP-2 and LMP-7 have likewise been documented in a number of tumor types [30,59]. Also tumors and/or their surrounding stroma may produce immunosuppressive factors like TGF-β, IL-10 and vascular endothelial growth factor (VEGF) that can induce production of immature myeloid cells and regulatory T-cells (Tregs) that inhibit dendritic cell maturation and activation of T-cells in a tumor-specific immune response [37]. Thus immunotherapeutic strategies of OVCA based on augmentation of humoral immune responses against specific TAA must consider creating immune-favorable conditions in OVCA patients by abrogating the immune suppressive effects of the tumor. Several clinical trials based on the usage of monoclonal antibodies to known OVCA tumor antigens are discussed below.

Oregovomab is a murine monoclonal antibody (Mab) that strongly binds to CA125, forming immune complexes that is recognized by immune effector molecules, most importantly T-cells that can induce both humoral and cellular immune responses against OVCA. A pilot

phase II study conducted by Ehlen et al. examined clinical and immunologic effects of oregovomab in pre-treated patients who had recurrent OVCA [17]. Thirteen OVCA patients, stage (III–IV) were enrolled in their study who had recurrent or progressive disease with elevated CA125 (range (50.5– 26000 U/mL). All the patients had been previously treated with chemotherapy and the median time from prior chemotherapy to initiation of systemic administration of oregovomab (2 mg) was 2.5 months (range 1.4–37.8 months). Both humoral responses to anti-idiotypic antibody and CA125, and T-cell responses to CA125-orogovomab were observed in more than 50% of patients. Treatment was well tolerated and stabilization of disease and survival for more than 2 years was observed in 3/13 patients and it was associated with robust immune responses, thereby indicating the immunologic activity and safety of oregovomab in the treatment protocol for recurrent OVCA. In 2004, Berek et al. initiated a randomized clinical trial of 145 patients with stage (III–IV) OVCA who were treated with oregovomab or placebo administered at weeks 0, 4, and 8, and every 12 weeks for up to 2 years or until recurrence [5]. Follow-up surveys for up to 5 years from randomization were collected for the same study population that included 145 OVCA patients to evaluate long-term outcomes for this patient population [6]. The relation of time-to-release, survival post-relapse and overall survival was analyzed. Median survival time for patients who were treated with oregovomab was 57.5 months and for those who were treated with placebo was 48.6 months (hazard ratio = 0.72; 95% confidence interval, 0.41–1.25). Their study indicated that the rate of CA125 increase at relapse was highly significant predictor of survival after relapse. In a different clinical study, Mobus et al. evaluated the therapeutic value of the murine monoclonal antibody B43.13 (immunoglobulin G1 antibody to CA125) in treating recurrentOVCA patients. Forty four patients who were enrolled in their study had prior treatment composed of surgery platimum-based chemotherapy and had CA125 value that exceeded 35 U/mL at some time during the course of the disease. These patients were treated with intravenous administration of technetium 99m–labeled monoclonal antibody-B43.13 and humoral immune responses were evaluated against human antimurine antibodies (HAMA), against B43.13 variable region (Ab2) and against antibodies to CA125 antigen itself. Their study indicated that 27/40 (67.5%) were HAMA responders, 20/23 (87%) of patients exhibited both HAMA and Ab2 responses and of 32 patients, 28% showed > 3-fold increase in anti-CA124 antibody level over the baseline. After the first dose of B43.13, 56.8% patients survived for more than 12 months and 34.1% patients survived for more than 24 months. Median survival time increased 3-fold for HAMA respon-ders (22.6 months) versus non-responders (7.2 months; $P < 0.0016$, log-rank test) and 2-fold for Ab2 responders (18.3 months) versus non-responders (9.3 months). Their study indicated that the treatment was well tolerated with no adverse side effects and B43.13 treatment did elicit multiple immune responses that were directly associated with improved clinical outcomes [51].

Pertuzumab is a recombinant, humanized monoclonal antibody directed against human epidermal growth factor receptor 2 (Her2) that inhibits ligandactivated heterodimerization with other Her receptors, mostly Her3 [18]. In the phase II, randomized, placebo-controlled, double-blind clinical trial, Makhija et al. showed that a combination of gemcitabine and pertuzumab may be beneficial for the treatment of platinum-resistant OVCA. One hundred and thirty patients were enrolled in this study who had platinum-resistant or platinum-refractory cancer and were randomly assigned in a 1:1 ratio of gemcitabine (800 mg/m^2 on days 1 and 8 of a 21 day cycle) plus either placebo or pertuzumab (840 mg loading dose, followed by 420 mg every 3 weeks). In patients whose tumors had low Her3 mRNA expression (< median, $n = 61$), longer progression free survival (PFS) was observed for those who were treated with gemcitabine plus pertuzimab arm compared with the gemcitabine alone (PFS hazard ratio = 0.32; 95% CI, 0.17 to 0.59; $P = 0.0002$) [46].

A clinical study by Hurt et al. demonstrated that a combination of paclitaxel and bevacizumab (a humanized anti-VEGF monoclonal antibody) in the treatment protocol improved PFS in patients with recurrent OV-CA. Fifty five patients with recurrent EOC, primary peritoneal carcinoma or fallopian tube cancers were enrolled in this study who received combination paclitaxel and bevacizumab (PB). The overall response rate was 60%. Complete response was observed in 25% of patients (range 5–38 months; median PFS 14 months) and a partial response rate was noted in 35% of patients (range 2–15; median PFS 5 months). The authors concluded that a combination of PB exhibited an acceptable toxicity profile and increased the response rate and PFS in patients with recurrent OVCA [29].

In 2008, Oei et al. conducted a randomized phase III clinical trial in which they investigated whether induction of MUC1 antibodies using yttrium-90-murine IgG1 monoclonal human milk fat globule 1 (HMFG1) MUC1 antibody was related to survival of EOC patients. Four hundred and forty four patients with EOC stage (Ic-IV) and macrosopically negative second-look laparoscopy following initial debulking and 6 courses of platinum-based chemotherapy were enrolled in their study. These patients were randomized between standard treatment (ST) plus intraperitoneal (IP) ^{90}Y- HMFG1 (active treatment, AT) and ST and serum samples from 208 patients in the AT arm and 199 patients in the ST arm were tested for IgG antibodies to MUC1. Anti-MUC1 IgG at weeks 4, 8 and 12 ranked higher in the AT arm than ST arm ($P < 0.001$). Univariate analysis showed that anti-MUC1 IgG was associated with a benefit in overall survival (OS) and disease-free survival (DFS) for the patients in the AT arm. Their results indicated that immunotherapy against MUC1 could be effective in the treatment of EOC [55].

Lastly, in a preclinical study, Richter et al., demonstrated that high-grade chemotherapy resistant ovarian carcinoma cell lines that showed high EpCAM surface expression were sensitive to MT201 (adecatumab, a humanized antibody to EpCAM) antibodydependent cell-mediated cytotoxicity resulting in cell death (range of killing, 27–66%). Their results indicated that MT201 may represent a novel immunotherapy for more aggressive OVCA that are resistant to chemotherapy [58].

Thus, humoral immune responses directed against tumor antigens in OVCA patients play a significant role in strategic design of powerful immunomodulatory tumor specific antibodies and create new opportunities in ovarian cancer therapeutics. Following tumor debulking and first-line chemotherapy, immunothera-py may augment the pre-existing anti-tumor immune responses to eradicate the residual micrometastatic tumor and may prevent OVCA recurrence thereby improving survival outcomes in OVCA patients.

3. Conclusions

The immunogenic nature of OVCA is a promising property of this cancer that can be exploited for the identification of large number of tumor antigens involved in the pathogenesis of OVCA. This tumor im-munogenicity leads to the generation of large diversity of antibody repertoire directed against autologous tumor-related antigens. The tumor autoantibodies have been shown to have diagnostic value for the detection of OVCA at early stage when the tumor burden is low. Although in this review we discussed the use of autoantibodies to single TAA for early detection of cancer, panels of tumor autoantibodies may provide better sensitivity and specificity for diagnostic screening tests [43]. The screening strategy using tumor autoantibodies as biomarkers represents a simple non-invasive immunoassay that can be essentially performed at a minimal cost. Because 60% patients with advanced stage ovarian carcinoma have been shown to relapse while they are on first-line chemotherapy treatment, there is an urgent need to identify biomarkers that could predict the likelihood of OVCA recurrence at the time of diagnosis. Monitoring of

OVCA during first-line chemotherapy treatment rather than after the completion of the chemotherapy using tumor autoantibody titers may be a more informative way to predict early recurrence that generally leads to more aggressive platinum-refractory OVCA. This will be highly advantageous for the OVCA patients because these patients can have tailored chemotherapy regimen that will result in better survival. Reports from different laboratories confirmed the direct correlation between the level of tumor antibodies against a particular TAA and i) the risk of OVCA recurrence and ii) patients survival outcome in OVCA. Antitumor immune responses in OVCA play an important role in the development of immunotherapeutic strategies and the immunogenicity of OVCA has been utilized in antibody-based immunotherapy. Immunotherapy using radio-labeled or unlabeled murine Mab that are specific for known OVCA tumor antigens with or without the use of chemotherapeutic agents have shown great promise in randomized clinical trials. As conventional therapy like surgery and chemotherapy have been shown to have immunosuppressive effects at certain circumstances [7,27], therefore there is a need for immunological intervention of the treated patients during or after chemotherapy. The boosting of immune response by lowering the toxic effects of chemotherapy can be achieved by several ways for example, i) by using drugs that are synthetic versions of substances produced naturally in the body, like proleukin, an artifical form of interleukin-2, that helps in the activation of T-cells; Neupogen are version of natural substances called colony stimulating factors, that helps in the formation of new white blood cells in the bone marrow[2]; ii) by using insulin potentiation therapy (IPT) that sensitizes cancer cells to chemotherapy because cancer cells have more insulin receptors on cell surface and uptake of glucose along with the chemotherapeutic agent will be more in cancer patient treated first with insulin that lowers the blood glucose level[3]; iii) by using dialyzable leucocyte extract (DLE), an immunological agent used as an adjuvant in chemotherapy that resulted in significant immunological recovery in breast cancer patient who underwent heavy chemotherapy[4]. Encouraging results of various clinical trials using Mab-based immunotherapy of OV-CA indicate that Mabs may have potential to complement current first-line chemotherapy treatment that ultimately may bring a significant improvement to overall survival of OVCA patients.

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Table 1

Diagnostic and prognostic values of autoantibodies to various TAAs in OVCA

TAA AAbs	Detection method	Study population	Percentage of AAb titer	Clinical value Diagnostic/prognostic utility	References
p53	ELISA	EOC stage (I–II) (*n* = 30)	10%	Serum p53 AAbs have limited clinical value.	[19]
		EOC stage (III–IV) (*n* = 56)	26.80%		
p53	ELISA	Invasive OVCA (*n* = 113)	19%	Serum p53 AAbs correlated well with aggressive cancer.	[69]
		Borderline tumors (*n* = 15)	0%		
		Benign tumors (*n* = 117)	0%		
p53	ELISA	Serous cases (*n* = 60, stage (III–IV))		Serum p53 AAbs were associated with improved survival, (hazard ratio = 0.57; (95% CI), 0.33–0.97; *P* = 0.04). p53 AAbs have prognostic utility.	[3]
p53	ELISA	OVCA late stage (*n* = 104)		p35 AAbs correlated well with better survival. The median survival of patients with p53 AAbs was 51 months compared to patients without p53 AAbs. p53 AAbs have prognostic value.	[23]
p53	ELISA	OVCA late stage (*n* = 44)		After first-line chemostherapy, pathological complete response was not achieved by patients with p53 AAbs compared to 24.1% patients without p53 AAbs. p53 AAbs had no prognostic relevance to progression free survival.	[19]
p53	ELISA	OVCA (*n* = 83)	46%	Bivariate analyses showed patients with p53 AAbs had a 1.96-fold risk of OVCA relapse (95% CI, 1.02–3.78)	[69]
MUC1	ELISA	Benign and malignant ovarian tumors Healthy: pregnant and non-pregnant women		Level of MUC1 AAbs were much lower than healthy (*P* < 0.0001). Univariate analysis revealed association of greater survival in OVCA (*P* = 0.015)	[57]
65 antigens	Epitomics	Invasive OVCA		Sensitivity = 55%	[8]
		stage I (*n* = 20)		Specificity = 98%	
		Borderline OVCA (*n* = 3)		Serum AAbs to 65 antigens may have diagnostic value for early detection of OVCA in high risk population.	
		OVCA late stage (*n* = 46)			
		Healthy (*n* = 60)			
13 antigens	ELISA	OVCA (*n* = 32)		Sensitivity = 62.5%	[40]
	Immunoblot	Healthy (*n* = 82)		Specificity = 85.4%	
				Have diagnostic value for early detection of OVCA.	

TAA AAbs	Detection method	Study population	Percentage of AAb titer	Clinical value Diagnostic/prognostic utility	References
HOXB7	SEREX	OVCA (*n* = 39)	33.30%	HOXB7 AAbs have diagnostic value for early diagnosis of OVCA.	[53]
	ELISA	Healthy (*n* = 29)	0.03%		
HSP-90	ELISA	OVCA stage (I–II) (*n* = 10)	10%	HSP-90 AAbs were present mostly in late stage OVCA.	[45]
		OVCA stage (III–IV) (*n* = 22)	32%		
		Colorectal cancer (*n* = 37)	3%		
		Breast cancer (*n* = 13)	8%		
		Lung cancer (*n* = 10)			
		Benign gynecologic disease (*n* = 20)	5%		
		Benign breast lesions (*n* = 10)			
		Healthy (*n* = 20)			
STIP-1	2D–DITA	OVCA (*n* = 63)		High plasma STIP-1 AAb titer in serous OVCA compared to other histological subtypes. STIP-1 AABs have diagnostic value for early detection of OVCA.	[35]
	ELISA	Borderline ovarian tumors (*n* = 13)			
		Healthy (*n* = 63)			
NY-ESO-1/ LAGE-1 (CT Ag)	ELISA	OVCA stage (IIIC, papillary serous) (*n* = 37)	30%	Detectable serum AAbs were present in OVCA patients for up to 3 years after initial diagnosis. Candidate biomarker for early detection of OVCA.	[54]
SPAG9 (CT Ag)	ELISA Immunoblot	Serous adenocarcinoma (*n* = 19)	58%	SPAG9 AAbs may act as a candidate biomarker for early diagnosis of OVCA.	[22]
		Clear cell carcinoma (*n* = 2)	100%		
		Mucinous adenocarcinoma (*n* = 3)	100%		
		Various histotypes early stage (I–II) (*n* = 8)	62.50%		
		OVCA late stage (III–IV) (*n* = 22)	68%		
IL-8	Luminex	OVCA early stage (I–II) (n-44)		Sensitivity = 65.5%	[42]
		OVCA late stage (*n* = 50)		Specificity = 98%	
		Benign pelvic masses (*n* = 37)		Serum IL-8 AAbs have diagnostic value for early diagnosis of OVCA.	
		Healthy (*n* = 80)			
MUC1	ELISA	EOC (serous borderline, serous invasive, mucinous, endometroid, and other/undiff) (*n* = 668)	OD >= 0.6, 45.8% OD >= 1.0, 25%	Plasma MUC1 AAbs may act a biomarker for early detection of OVCA.	[12]
		Healthy (*n* = 721)	OD >= 0.6, 33.8% OD >= 1.0, 12.3%		
S100A7	2D–DITA	OVCA early stage (*n* = 23)		Significant difference of S100 A7 AAb level	[20]

TAA AAbs	Detection method	Study population	Percentage of AAb titer	Clinical value Diagnostic/prognostic utility	References
	ELISA	Late stage OVCA ($n = 69$) Benign gynecologic disease ($n = 11$) Healthy ($n = 35$).		between early and late stage OVCA compared to healthy. (Mann -Whiteney U test ($P = 0.05$ and $P < 0.001$)	

AAbs = Autoantibodies.
Ag = Antigen.