Published in final edited form as:

Gynecol Oncol. 2011 September ; 122(3): 560–566. doi:10.1016/j.ygyno.2011.05.039.

Comparison of Candidate Serologic Markers for Type I and Type II Ovarian Cancer

Dan Lu¹, Elisabetta Kuhn¹, Robert E. Bristow², Robert L. Giuntoli II², Susanne Krüger Kjær³, le-Ming Shih^{1,2}, and Richard B.S. Roden^{1,2,#}

¹ Department of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD

² Department of Gynecology and Obstetrics, and Oncology, Johns Hopkins University School of Medicine, Baltimore, MD

³ Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark

Abstract

Objective—To examine the value of individual and combinations of ovarian cancer associated blood biomarkers for the discrimination between plasma of patients with type I or II ovarian cancer and disease-free volunteers.

Methods—Levels of 14 currently promising ovarian cancer-related biomarkers, including CA125, macrophage inhibitory factor-1 (MIF-1), leptin, prolactin, osteopontin (OPN), insulin-like growth factor-II (IGF-II), autoantibodies (AABs) to eight proteins: p53, NY-ESO-1, p16, ALPP, CTSD, B23, GRP78, and SSX, were measured in the plasma of 151 ovarian cancer patients, 23 with borderline ovarian tumors, 55 with benign tumors and 75 healthy controls.

Results—When examined individually, seven candidate biomarkers (MIF, Prolactin, CA-125, OPN, Leptin, IGF-II and p53 AABs) had significantly different plasma levels between type II ovarian cancer patients and healthy controls. Based on the receiver operating characteristic (ROC) curves constructed and area under the curve (AUC) calculated, CA125 exhibited the greatest power to discriminate the plasma samples of type II cancer patients from normal volunteers (AUC 0.9310), followed by IGF-II (AUC 0.8514), OPN (AUC 0.7888), leptin (AUC 0.7571), prolactin (AUC 0.7247), p53 AABs (AUC 0.7033), and MIF (AUC 0.6992). p53 AABs levels exhibited the lowest correlation with CA125 levels among the six markers, suggesting the potential of p53 AABs as a biomarker independent of CA125. Indeed, p53 AABs increased the AUC of ROC curve to the greatest extent when combining CA125 with one of the other markers. At a fixed specificity of 100%, the addition of p53 AABs to CA125 increased sensitivity from 73.8% to 85.7% to discriminate type II cancer patients from normal controls. Notably, seropositivity of p53 AABs is comparable in type II ovarian cancer patients with negative and positive CA125, but has no value for type I ovarian cancer patients.

Conclusions—p53 AABs might be a useful blood-based biomarker for the detection of type II ovarian cancer, especially when combined with CA125 levels.

© 2011 Elsevier Inc. All rights reserved.

#Corresponding author: Richard Roden, Johns Hopkins University, Cancer Research Building 2, Room 308, 1550 Orleans St, Baltimore, MD 21231 USA. Tel: 410 502 5161; Fax: 443 287 4295; roden@jhmi.edu.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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

INTRODUCTION

Early detection of ovarian cancer is associated with an improved outcome. However there is no sufficiently predictive screening test, and consequently the majority of cases present at an advanced stage. Despite aggressive surgery and chemotherapy regimens, ovarian cancer remains the most lethal gynecologic malignancy. Ovarian cancer is not a single disease, but comprises an amalgam of tumor types with distinct pathogenesis and morphologic features. Screening tests should be developed to detect a single entity, preferably of greatest medical significance, rather than a heterogeneous conglomeration of neoplasms. It was recently proposed that ovarian cancer can be grouped into two broad categories designated type I and type II tumors based upon their distinct pathogenesis [1]. Type I tumors are relatively genetically stable and generally exhibit an indolent behavior. In contrast, type II tumors are highly aggressive and almost always have progressed to advanced stage at diagnosis, when current available therapies are seldom curative [2]. Type II tumors constitute approximately 75% of ovarian cancer but are responsible for 90% of ovarian cancer deaths. Thus a screening test is urgently needed for low volume type II tumors using sensitive and specific biomarkers detectable before the disease is clinically manifest, or more ideally prior to metastasis [1].

High grade serous carcinoma (HGSC) is by far the most common type II ovarian cancer. This subgroup of ovarian cancers exhibits genetic instability and more than 80% [3, 4] of the tumors carry mutation in *TP53* gene [5]. Morphologic and recent molecular genetic studies suggest that the majority of the HGSCs arise from the epithelium of fallopian tube rather than the ovarian surface epithelium as previously thought [6–11]. p53 mutation and subsequent protein accumulation are likely early events in the development of HGSC, since positive p53 immunostaining is observed in serous tubal intraepithelial carcinoma (STIC), the proposed precursor of HGSC [12, 13].

The pathogenesis of cancer from a normal cell to life-threatening metastatic tumor masses is associated with a series of genetic and epigenetic changes that result in mutant or abnormally expressed gene products [14–16]. These gene products can potentially be shed into the body fluid as observed for the surface glycoprotein CA125, which is elevated in the blood of most ovarian cancer patients [17–19]. In addition, aberrant gene products are potentially antigenic and can be recognized by the humoral immune system to generate specific antibody responses [20, 21], for example, mutated p53 or aberrant expression of NY-ESO-1 triggers autoantibodies (AABs) in a significant fraction of cancer patients [22–25]. Notably, p53 AABs can appear before the clinical diagnosis of lung cancer [24, 26], suggesting its potential as a biomarker for the early detection of ovarian cancer or even its precursors.

The most extensively studied biomarker of ovarian cancer, CA125, was licensed for monitoring recurrence only. Although an increasing number of novel biomarkers for ovarian cancer have been proposed with advances in the technologies of genomics and proteomics, no single marker has proven sensitive and specific enough for clinical application in screening for ovarian cancer. As compared to a single marker, combining different markers as a panel potentially increases predictive value for detecting ovarian cancer when it is considered as a single entity [27, 28]. Therefore, in this report, we analyzed 14 promising candidate biomarkers for ovarian cancer, each selected based on promising earlier studies, aiming to identify the optimal combination panel for discriminating type II ovarian cancer patients from normal controls and to explore their value for detecting type I disease.

MATERIALS AND METHODS

Human plasma samples

Study individuals were recruited at the Johns Hopkins Hospital and included 151 patients with ovarian carcinoma and three groups of controls: 23 with borderline ovarian tumors, 55 with benign tumors, and 75 healthy female blood donors. The characteristics of the recruited patients were summarized in table 1. Blood of tumor patients was collected into heparin-treated tubes prior to surgery, plasma obtained and stored at -80°C . Written informed consent was provided by each participant and this study was approved by the Johns Hopkins Institutional Review Board.

Enzyme-linked immunosorbent assay (ELISA)

The levels of p53 AAbs in the plasma were measured using the commercial p53 ELISA^{PLUS} (Autoantibody) kit from Calbiochem (QIA53) following the manufacturer's instructions. To measure the levels of AAbs to NY-ESO-1 and p16, hexahistidine (6His)-tagged fusion proteins of NY-ESO-1 and p16 were expressed in Rosetta DE3 cells (Novagen) using pET28a-NY-ESO-1 (NM_139250.1; GI: 21281684) and pET28a-p16 (NM_000077.4; GI: 300863097) constructs followed by purification as described previously [25]. The quality of each protein was examined with SDS-PAGE followed by Coomassie staining and western blot. The protein concentrations were estimated with absorbance at 280 nm (A_{280}) using Nanodrop. Maxisorp immuno plates (Nunc, Rochester, NY) were coated with 0.1 $\mu\text{g}/\text{well}$ of the purified protein at 4°C overnight (O/N). After being blocked with 1% (w/v) BSA in PBS for 1 hr at room temperature (RT), the plates were incubated with human plasma samples diluted at 1:50 in the blocking buffer for 1 hr at RT. Following extensive wash with PBS/0.01% (v/v) Tween-20, the plates were incubated with HRP-conjugated sheep anti-human IgG (GE healthcare) diluted at 1:5000 in the blocking buffer for 1 hr at RT. The plates were washed again, and then 1-StepTM ABTS substrate (Pierce) was added for 15 min at 37°C for color development. The absorbance was measured at 405 nm (A_{405}) using a Benchmark Plus ELISA plate reader (Bio-Rad, Hercules, CA). Commercial antibodies were used as positive controls in the assay.

Sandwich ELISA was used to detect AAbs to ALPP (NM_001632.3; GI: 94721245), CTSD (NM_001909.3; GI: 23110949), B23 (NM_002520.4; GI: 262331543), GRP78 (NM_005347.2; GI: 21361242), and SSX (NM_005635.2; GI: 28559010). Myc-DDK-tagged proteins were expressed in 293TT cells using the Human cDNA ORF Clones for each protein (OriGene). The expression was confirmed by western blot using anti-Myc and anti-DDK antibodies. Maxisorp immuno plates (Nunc, Rochester, NY) were coated with 500ng/well of anti-DDK antibody (OriGene) at 4°C O/N. After wash, the plates were blocked with 5% (w/v) milk in PBS for 3 hr at RT. And then 100 $\mu\text{g}/\text{well}$ of lysate from 293TT cells expressing individual protein was added for incubation at 4°C O/N to capture the protein onto the plates. To eliminate the influences of nonspecific binding due to plasma, the lysate from 293TT cells transfected with the empty vector was added to separate plates serving as background control. The plates were incubated with human plasma samples diluted at 1:100 in the blocking buffer for 1 hr at RT following extensive wash. The bound AAbs from the plasma were detected using HRP-conjugated anti-human IgG (GE healthcare) and 1-StepTM ABTS substrate (Pierce) as described above. Commercial antibodies (ALPP: ab16695, abcam; CTSD: 219361, Calbiochem; B23: ab24412, abcam; GRP78: sc-1050, Santa Cruz; SSX: sc-28697, Santa Cruz) against each individual protein were used as positive controls in the assay. The relative level of AAbs to each protein in individual plasma was calculated by subtracting the A_{405} of background control from the A_{405} of the corresponding protein.

MILLIPLEX™ MAP assay

The MILLIPLEX™ MAP Human Cancer Biomarker Panel kit (Millipore) was used to measure the levels of MIF, Leptin, Prolactin, CA-125, OPN, and IGF-II in human plasma according to the manufacturer's protocol. The plates were washed with Bio-Plex Pro II Wash Station (Bio-Rad, Hercules, CA). The samples were read with Bio-Plex array reader (Bio-Rad, Hercules, CA) and the data was analyzed with Bio-Plex Manager Software 5.0.

Statistical analysis

Comparison between different disease statuses for individual markers was done with the Wilcoxon two-sample test and t test after log transformation, difference is considered significant when passing both tests. The ability of each single marker to discriminate disease from control was assessed with receiver operating characteristic (ROC) curve generated through a univariate logistic regression model, and the area under curve (AUC) was measured based on generalized U-statistic. The discrimination ability of combined markers was assessed with weighted linear combination of markers using a multiple logistic regression model. The adequateness of the multiple logistic regression model was assessed by the Hosmer-Lemeshow goodness-of-fit test. The Akaike information criterion (AIC) and generalized coefficient of determination were used to compare multiple logistic regression model and univariate logistic regression model.

Comparison between different markers and/or combinations for discrimination ability was tested with asymptotic normal approximation [29]. The correlation between two different markers was made with Spearman rank-order correlation test. Statistical Analysis Software 9.2 (SAS Institute, Inc., Cary, North Carolina) was used in all analyses. Statistical significance was set at p value less than 0.05.

RESULTS

Levels of individual markers by disease status

The candidate markers were selected based on previous publications suggesting their promise for detection of 'ovarian cancer' [19, 25, 30–32]. They include MIF, Leptin, Prolactin, CA-125, OPN, IGF-II, and autoantibodies (AAbs) to eight proteins: p53, NY-ESO-1, p16, ALPP, CTSD, B23, GRP78, and SSX. To simplify the analysis of combination of markers for the detection of type II ovarian cancer, we first examined whether these markers individually exhibited differential levels in the plasma of patients with different disease status. After analyzing the data with different statistical models (Wilcoxon two-sample test and t test after log transformation), we found that among the fourteen markers examined, the levels of six candidate markers (AAbs to p16, ALPP, CTSD, B23, GRP78, and SSX) were similar among different groups of patients (data not shown) and were not analyzed further. In contrast, the levels of five markers (MIF, prolactin, CA-125, OPN, and AAbs to p53) were consistently increased while the levels of two markers (leptin and IGF-II) were consistently decreased in the plasma of patients with type II ovarian cancer as compared to healthy controls (figure 1). Although AAbs to NY-ESO-1 exhibited higher levels in the plasma of patients with type II tumor, the difference reached significance in only one of the two statistical tests. Likewise, NY-ESO-1 AAbs were not observed in the plasma of type I ovarian cancer patients.

We examined the impact of age on individual biomarkers by comparing their levels in different age groups in type II cancer patients. Using median age 59 as a cut-off, we found that patients of 59 and younger contained similar levels of the individual markers as the older patients (not shown). When we tested the influence of age on the biomarkers in benign tumor group which carry the closest levels of the biomarkers to normal controls, using

Spearman rank-order correlation test; we found again that age did not have any effect on the levels of the biomarkers.

Except for the levels of AAbs to p53 and MIF which were comparable in the plasma of patients with type I tumor and healthy controls, the levels of the other five markers (prolactin, CA125, leptin, OPN, and IGF-II) in the plasma of patients with type I tumor showed similar trend as those with type II tumor when compared to the plasma of healthy controls (figure 1). These data suggest that p53 AAbs and MIF are specific for type II ovarian cancer.

When we compared the plasma levels of these seven markers in patients with type I and type II tumors, we found that type II tumor patients carried higher levels of p53 AAbs and CA125 but lower levels of IGF-II. Patients with the two different types of tumors had comparable levels of MIF, prolactin, leptin, and OPN in their plasma (figure 1).

To compare the performance of the seven candidate markers that are potentially associated with type II ovarian cancer, we then plotted a receiver operating characteristic (ROC) curve and calculated the area under curve (AUC) for each marker using healthy blood donors as controls. As shown in figure 2, CA125 exhibited the greatest ability to discriminate type II cancer patients from the healthy controls based on the AUC (0.9310), followed by IGF-II (0.8514), OPN (0.7888), leptin (0.7571), prolactin (0.7247), p53 AAbs (0.7033), MIF (0.6992).

Analysis of combined markers

We then focused on the seven candidate markers demonstrating significant differences between type II ovarian cancer patients and normal controls because the most optimal combination panel should come from these markers. The best combination panels of multiple (from 2 to 6) markers based on ROC and AUC analyses always include CA125. Interestingly, the combination of CA125 and p53 AAbs showed the best discrimination performance among all the two-marker panels although p53 AAbs had the second-lowest value of AUC as a single marker (table 2). The AUC increased significantly from 0.9310 for CA125 alone to 0.9694 for combining p53 AAbs with CA125 ($p=0.0122$). In contrast, the increase in AUC by the second-best combination CA125 and leptin was not statistically significant ($p=0.095$). When using a cut-off of 9 U for p53 AAbs and 35 IU/ml for CA125, this two-marker panel was able to yield a sensitivity of 85.7% at 100% specificity with our patient samples, compared to a sensitivity of 73.8% at the same specificity for CA125 alone. The six-marker panel of MIF, prolactin, leptin, OPN, IGF-II and CA125 also significantly improved the performance with an AUC of 0.9719 when compared to CA125 alone. However, this is not significantly different from the two-marker panel combining p53 AAbs and CA125 ($p=0.8407$), suggesting that combination of p53 AAbs and CA125 might be a similarly effective marker for type II ovarian cancer when compared to the six-marker panel.

Independence of AAbs and CA125 in type II ovarian cancer patients

Since p53 AAbs improved the performance of distinguishing type II ovarian cancer patients from normal controls to a greater extent compared to the other markers (MIF, prolactin, leptin, OPN, IGF-II) tested when combined with CA125 despite the fact that p53 AAbs failed to perform as well as the others as a single marker, we hypothesized that the production of p53 AAbs is independent of the detection of CA125 in ovarian cancer patients. When we compared the correlation of CA125 with the other markers of interest in the plasma of the type II ovarian cancer patients, we found that the level of p53 and NY-ESO-1 AAbs have the least correlation with CA125 (table 3).

To further evaluate the independence of p53 AAbs from CA125, we compared the generation of p53 AAbs in ovarian cancer patients with different levels of CA125 (figure 3). When we limited our analysis to the patients with type II ovarian cancer, the level of p53 AAbs were not statistically different between the plasma of cancer patients with negative and positive CA125 using a cut-off of 35 IU/ml for CA125, although it seems that the absolute levels of p53 AAbs are relatively lower in the CA125 < 35 IU/ml patients. When we used a cut-off of 9 U for p53 AAbs, 15 out of 33 (45.5%) patients with negative CA125 were positive for p53 AAbs and 29 out of 93 (31.2%) patients with positive CA125 were positive for p53 AAbs. Again, there is no statistical difference in the seropositivity of p53 AAbs between type II ovarian cancer patients with different levels of CA125 ($p=0.1395$).

We also explored whether NY-ESO-1 AAbs in type II ovarian cancer patients were independent of CA125 levels (Figure 3). When using a cut-off of 35 IU/ml for CA125, there was no statistically significant difference in the presence of NY-ESO-1 AAbs; plasma from 5 of 33 (15.2%) type II ovarian cancer patients with CA125 < 35 IU/ml contained detectable NY-ESO-1 antibodies, whereas 17 of 93 (18.3%) type II ovarian cancer patients with CA125 > 35 IU/ml exhibited NY-ESO-1 AAb. However, the detection of AAbs to NY-ESO-1 in the plasma of patients with type II ovarian cancer was independent of AAbs to p53 (figure 3).

DISCUSSION

In this report, we confirmed that seven previously reported blood biomarkers (CA125, MIF-1, leptin, prolactin, OPN, IGF-II, and p53 AAbs) are associated with the presence of ovarian cancer [19, 25, 30]. We further demonstrated that among them, seropositivity of p53 AAbs and elevated MIF are associated with type II, not type I ovarian cancer; while the other five are not type-specific markers for ovarian cancer, although the blood levels of CA125 and IGF-II exhibit more dramatic change in type II than type I tumor patients when compared to healthy controls (figure 1). The specificity of p53 AAbs for type II tumors supports the notion that 'ovarian cancer' is not a single disease, requiring different biomarkers be utilized for each specific disease type. Similarly, NY-ESO-1 AAbs were not apparent in the plasma of type I ovarian cancer patients, but they were detected in a small fraction of type II ovarian cancer patients. Notably, type II tumors are more aggressive and prevalent than type I tumors and need blood biomarkers to aid the early detection [10], therefore it is reasonable to focus on type II tumors for the identification of biomarkers in the future.

In theory, the efficient combination of biomarkers should consist of biomarkers that are not correlated with each other because the combined effect of such markers will be closer to an additive effect. Through the statistical analysis of currently promising candidate markers, our study suggests that seropositivity of p53 AAbs might be a useful blood marker to combine with CA125 for the early detection of type II ovarian cancer. This notion was supported by the low level of correlation between the production of p53 AAbs and CA125 in type II ovarian cancer patients (table 3), and by the comparable seropositivity of p53 AAbs in the patients with negative and positive CA125 (figure 3). These data clearly indicated the additional benefit of combining p53 AAbs with CA125 over CA125 alone in the detection of type II ovarian cancer. Since CA125 positivity correlates with increased ovarian cancer volume measured under laparoscopy/laparotomy [19], it suggests that the production of p53 AAbs by type II ovarian cancer patients is relatively independent of the extent of disease. A similar observation was made for NY-ESO-1 antibodies, suggesting that AAbs can be triggered by low volume type II ovarian cancer. Indeed, p53 AAbs were previously shown to be present in sera of patients with asbestosis several years prior to a diagnosis of cancer [26], therefore it is highly possible that immune system recognizes limited amount of mutant

p53 accumulation occurring early in the development of ovarian cancer [12, 13] and mount a well-adapted humoral immune response by generating detectable levels of p53 AAbs early enough when other markers are not detectable [33]. We found that although the magnitude of the p53 AAb responses tended to be lower in CA125 negative patients, the percentage of p53 AAb seropositivity tended to be higher in this group of patients when we used a cut-off providing 100% specificity for p53 AAbs. However, we recognize that this result is only based on our small cohort study; and that much more work is needed in the future to evaluate how useful it is to combine p53 AAbs with CA125 in the early detection of type II ovarian cancer.

We found that recently identified markers MIF, leptin, prolactin, OPN, and IGF-II [30, 34] exhibited differential levels in the plasma of type II ovarian cancer patients compared to disease-free controls (figure 1); however, none of them behaved as well as CA125 as a single marker in our sample set based on AUC of ROC curve (figure 2). We observed a similar trend when we compared all the ovarian cancer patients with disease-free controls (data not shown). Similar to CA125, leptin, prolactin, OPN, and IGF-II also exhibited differential blood levels in patients with type I ovarian cancer and disease-free controls (figure 1), which may partially explain their association with ovarian cancers (i.e. presumably type I and type II), especially low stage disease, reported before [30, 34] since low stage disease is typically skewed more to type I tumors than high stage cases.

Previous studies [32, 35] identified significant seropositivity of IgGs against several other tumor associated antigens including NY-ESO-1, ALPP, CTSD, B23, GRP78, SSX in ovarian cancer patients, but not in normal controls or benign tumor patients. We did not detect a differential antibody response against ALPP, CTSD, B23, GRP78, and SSX with our patient cohort, but this may reflect the antigen source used in our immunoassay. Taylor *et al* used tumor-derived exosomal proteins in their assays, whereas we used recombinant tagged proteins expressed in 293TT cells and captured from detergent lysates. When Taylor *et al* compared the exosomal proteins and their recombinant counterparts produced in bacteria, the exosomal proteins exhibited much stronger immunoreactivity with the patient-derived antibodies [32], suggesting the importance of secondary modifications in AAb recognition of these antigens that are apparently not produced by over-expression in 293TT cells. Conversely, we have successfully used p53 and NY-ESO-1 expressed in 293TT cells or in bacteria to detect AAbs in ovarian cancer patients (data not shown).

In summary, our data suggests seropositivity of p53 AAbs as a biomarker relatively independent of CA125 for type II, but not type I, epithelial ovarian cancer. It is particularly useful in CA125 negative patients and combination of p53 AAbs and CA125 effectively improved the discrimination ability to detect type II ovarian cancer. Our results are based on comparison between plasma from women with and without clinically confirmed disease and should be extended to blood specimens from asymptomatic patients. Thus, combining the detection of p53 AAbs and CA125 in screening for type II ovarian cancer among an asymptomatic population warrants further study, but is unlikely to be helpful to identify patients with type I ovarian cancer. Further, the utility of p53 AAbs, CA125 and IGF-II as markers to discriminate between type II and type I in patients with low stage disease also needs further examination in independent and prospective studies.

Acknowledgments

Grant support was provided by the US PHS Grants RO1 CA122581 (RBSR, SKK), P50 CA098252 (RBSR, REB and RG), and the HERA foundation (DL). We thank the Tissue bank of the Johns Hopkins SPORC in Cervical Cancer for tissue specimens. We thank the HERA foundation for their encouragement and fund raising activities.

Abbreviations

HGSC	High grade serous carcinoma
AUC	area under curve
AIC	Akaike information criterion
AAbs	autoantibodies

BIBLIOGRAPHY

- Shih Ie M, Kurman RJ. Ovarian tumorigenesis: a proposed model based on morphological and molecular genetic analysis. *Am J Pathol.* 2004 May; 164(5):1511–8. [PubMed: 15111296]
- Kurman RJ, Visvanathan K, Roden R, Wu TC, Shih Ie M. Early detection and treatment of ovarian cancer: shifting from early stage to minimal volume of disease based on a new model of carcinogenesis. *Am J Obstet Gynecol.* 2008 Apr; 198(4):351–6. [PubMed: 18395030]
- Ayhan A, Kurman RJ, Yemelyanova A, Vang R, Logani S, Seidman JD, et al. Defining the cut point between low-grade and high-grade ovarian serous carcinomas: a clinicopathologic and molecular genetic analysis. *Am J Surg Pathol.* 2009 Aug; 33(8):1220–4. [PubMed: 19461510]
- Vang R, Shih Ie M, Kurman RJ. Ovarian low-grade and high-grade serous carcinoma: pathogenesis, clinicopathologic and molecular biologic features, and diagnostic problems. *Adv Anat Pathol.* 2009 Sep; 16(5):267–82. [PubMed: 19700937]
- Salani R, Kurman RJ, Giuntoli R 2nd, Gardner G, Bristow R, Wang TL, et al. Assessment of TP53 mutation using purified tissue samples of ovarian serous carcinomas reveals a higher mutation rate than previously reported and does not correlate with drug resistance. *Int J Gynecol Cancer.* 2008 May-Jun; 18(3):487–91. [PubMed: 17692090]
- Przybycin CG, Kurman RJ, Ronnett BM, Shih Ie M, Vang R. Are all pelvic (nonuterine) serous carcinomas of tubal origin? *Am J Surg Pathol.* 2010 Oct; 34(10):1407–16. [PubMed: 20861711]
- Kuhn E, Meeker A, Wang TL, Sehdev AS, Kurman RJ, Shih Ie M. Shortened telomeres in serous tubal intraepithelial carcinoma: an early event in ovarian high-grade serous carcinogenesis. *Am J Surg Pathol.* 2010 Jun; 34(6):829–36. [PubMed: 20431479]
- Sehdev AS, Kurman RJ, Kuhn E, Shih Ie M. Serous tubal intraepithelial carcinoma upregulates markers associated with high-grade serous carcinomas including Rsf-1 (HBXAP), cyclin E and fatty acid synthase. *Mod Pathol.* 2010 Jun; 23(6):844–55. [PubMed: 20228782]
- Folkins AK, Jarboe EA, Roh MH, Crum CP. Precursors to pelvic serous carcinoma and their clinical implications. *Gynecol Oncol.* 2009 Jun; 113(3):391–6. [PubMed: 19237187]
- Kurman RJ, Shih Ie M. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *Am J Surg Pathol.* 2010 Mar; 34(3):433–43. [PubMed: 20154587]
- Levanon K, Crum C, Drapkin R. New insights into the pathogenesis of serous ovarian cancer and its clinical impact. *J Clin Oncol.* 2008 Nov 10; 26(32):5284–93. [PubMed: 18854563]
- Folkins AK, Jarboe EA, Saleemuddin A, Lee Y, Callahan MJ, Drapkin R, et al. A candidate precursor to pelvic serous cancer (p53 signature) and its prevalence in ovaries and fallopian tubes from women with BRCA mutations. *Gynecol Oncol.* 2008 May; 109(2):168–73. [PubMed: 18342932]
- Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, et al. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: Evidence for a causal relationship. *Am J Surg Pathol.* 2007 Feb; 31(2):161–9. [PubMed: 17255760]
- Chin L, Gray JW. Translating insights from the cancer genome into clinical practice. *Nature.* 2008 Apr 3; 452(7187):553–63. [PubMed: 18385729]
- Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell.* 2000 Jan 7; 100(1):57–70. [PubMed: 10647931]
- Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. *Nat Med.* 2006 Aug; 12(8):895–904. [PubMed: 16892035]

17. Bast RC Jr, Klug TL, St John E, Jenison E, Niloff JM, Lazarus H, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med*. 1983 Oct 13; 309(15):883–7. [PubMed: 6310399]
18. Canney PA, Moore M, Wilkinson PM, James RD. Ovarian cancer antigen CA125: a prospective clinical assessment of its role as a tumour marker. *Br J Cancer*. 1984 Dec; 50(6):765–9. [PubMed: 6208925]
19. Jacobs I, Bast RC Jr. The CA 125 tumour-associated antigen: a review of the literature. *Hum Reprod*. 1989 Jan; 4(1):1–12. [PubMed: 2651469]
20. Old LJ, Chen YT. New paths in human cancer serology. *J Exp Med*. 1998 Apr 20; 187(8):1163–7. [PubMed: 9547328]
21. Stockert E, Jager E, Chen YT, Scanlan MJ, Gout I, Karbach J, et al. A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. *J Exp Med*. 1998 Apr 20; 187(8):1349–54. [PubMed: 9547346]
22. Bast RC Jr. Status of tumor markers in ovarian cancer screening. *J Clin Oncol*. 2003 May 15; 21(10 Suppl):200s–5s. [PubMed: 12743135]
23. Lubin R, Zalcman G, Bouchet L, Tredanel J, Legros Y, Cazals D, et al. Serum p53 antibodies as early markers of lung cancer. *Nat Med*. 1995 Jul; 1(7):701–2. [PubMed: 7585154]
24. Soussi T. p53 Antibodies in the sera of patients with various types of cancer: a review. *Cancer Res*. 2000 Apr 1; 60(7):1777–88. [PubMed: 10766157]
25. Tsai-Turton M, Santillan A, Lu D, Bristow RE, Chan KC, Shih Ie M, et al. p53 autoantibodies, cytokine levels and ovarian carcinogenesis. *Gynecol Oncol*. 2009 Jul; 114(1):12–7. [PubMed: 19398128]
26. Li Y, Karjalainen A, Koskinen H, Hemminki K, Vainio H, Shnaidman M, et al. p53 autoantibodies predict subsequent development of cancer. *Int J Cancer*. 2005 Mar 10; 114(1):157–60. [PubMed: 15523685]
27. Hussein N. Status of tumor markers in epithelial ovarian cancer has there been any progress? A review. *Gynecol Oncol*. 2011 Jan; 120(1):152–7. [PubMed: 20934205]
28. Moore RG, MacLaughlan S, Bast RC Jr. Current state of biomarker development for clinical application in epithelial ovarian cancer. *Gynecol Oncol*. 2010 Feb; 116(2):240–5. [PubMed: 19879639]
29. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the Areas under Two or More Correlated Receiver Operating Characteristic Curves: A Nonparametric Approach. *Biometrics*. 1988; 44:837–45. [PubMed: 3203132]
30. Mor G, Visintin I, Lai Y, Zhao H, Schwartz P, Rutherford T, et al. Serum protein markers for early detection of ovarian cancer. *Proc Natl Acad Sci U S A*. 2005 May 24; 102(21):7677–82. [PubMed: 15890779]
31. Piura B, Piura E. Autoantibodies to tumor-associated antigens in epithelial ovarian carcinoma. *J Oncol*. 2009; 2009:581939. [PubMed: 20145720]
32. Taylor DD, Gercel-Taylor C, Parker LP. Patient-derived tumor-reactive antibodies as diagnostic markers for ovarian cancer. *Gynecol Oncol*. 2009 Oct; 115(1):112–20. [PubMed: 19647308]
33. Finn OJ. Immune response as a biomarker for cancer detection and a lot more. *N Engl J Med*. 2005 Sep 22; 353(12):1288–90. [PubMed: 16177255]
34. Visintin I, Feng Z, Longton G, Ward DC, Alvero AB, Lai Y, et al. Diagnostic markers for early detection of ovarian cancer. *Clin Cancer Res*. 2008 Feb 15; 14(4):1065–72. [PubMed: 18258665]
35. Taylor DD, Atay S, Metzinger DS, Gercel-Taylor C. Characterization of humoral responses of ovarian cancer patients: antibody subclasses and antigenic components. *Gynecol Oncol*. 2010 Feb; 116(2):213–21. [PubMed: 19945743]

RESEARCH HIGHLIGHTS

We compared 14 biomarkers to discriminate the plasma samples of healthy volunteers and ovarian cancer patients.

Autoantibodies to p53 and NY-ESO-1 were present in plasma of type II but not type I ovarian cancer patients.

Combining p53 AAbs with CA125 significantly improved discrimination of plasma of type II cancer patients from that of healthy volunteers.

Figure 1. Levels of individual candidate biomarkers by disease type

The levels of autoantibodies specific to NY-ESO-1 (Panel A), autoantibodies specific to Tp53 (Panel B), MIF (Panel C), prolactin (Panel D), CA125, (Panel E), leptin (Panel F), OPN (Panel G) or IGF-II (Panel H) were examined in the plasma of patients with type II ovarian cancer (Type II), type I ovarian cancer (Type I), borderline ovarian tumors (Borderline), benign ovarian tumors (Benign), and the plasma of healthy volunteers (Healthy).

Figure 2. ROC analyses for each marker for discrimination between ovarian cancer patients and healthy volunteers

Receiver Operating Characteristic curves for each individual marker exhibiting differential levels between type II ovarian cancer patients and healthy volunteers (Panel A) or type I ovarian cancer patients and healthy volunteers (Panel B).

Figure 3. p53 and NY-ESO-1 AAbs in plasma of type II ovarian cancer patients with CA125 above and below normal values

Levels of p53 (Panel A) or NY-ESO-1 (Panel B) autoantibodies in the plasma of type II ovarian cancer patients with CA125 greater versus less than 35U/ml. Panel C shows a comparison of the levels of p53 and NY-ESO-1 AAb in type II ovarian cancer patients.

Characteristics of the patients.

Table 1

Type	Histotype	Stage					Menopause			Age (year)	
		I	II	III	IV	U/S ^a	pre	post	undclear	range	median
Type I	LGS [*]	2	1	6	1	0	5	5	0	26–65	40
	Endometrioid	3	2	1	0	0	3	3	0	32–79	51
	Clear Cell	3	0	0	1	0	3	1	0	32–55	41
	Mucinous	0	0	0	1	0	0	1	0	80	80
	Others	1	0	2	0	1	1	2	1	32–69	57.5
	Total	9	3	9	3	1	12	12	1	26–80	47
Type II	HGS ^{**}	1	3	85	30	0	23	88	8	35–83	59
	Mixed with HGS ^{**}	1	0	4	2	0	3	4	0	32–78	58
	Total	2	3	89	32	0	26	93	8	32–83	59
	Borderline	23					9	14	0	34–82	54
Benign		55					23	32	0	23–82	49

^{*} LGS: Low Grade Serous;
^{**} HGS: High Grade Serous;
^a U/S: Unstaged.

Table 2
AUC analysis of CA125 in combination with another marker

Area-under the curve (AUC) analysis when combining CA125 with one of the other candidate analytes for the discrimination between plasma samples of type II ovarian cancer and healthy volunteers.

Markers	AUC
CA125 alone	0.9310
CA125 + NYESO1 AAb	0.9374
CA125 + p53 AAb	0.9694*
CA125 + MIF	0.9358
CA125 + prolactin	0.9492
CA125 + leptin	0.9539
CA125 + OPN	0.9431
CA125 + IGF-II	0.9519

*
p=0.0122 for CA125 plus p53 AAbs versus CA125 alone.

Table 3
Correlation between candidate biomarkers and CA125

Spearman correlation coefficients (SCC) between CA125 and the other analytes were calculated.

Markers	SCC
CA125 vs. CA125	1.00000
CA125 vs. NYESO1 AAb	0.07360
CA125 vs. p53 AAb	0.16677
CA125 vs. MIF	0.36739
CA125 vs. prolactin	0.40211
CA125 vs. leptin	−0.33437
CA125 vs. OPN	0.48804
CA125 vs. IGF-II	−0.63529