

p53 Autoantibodies as Potential Detection and Prognostic Biomarkers in Serous Ovarian Cancer

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Abstract

Background: This study examined the value of serum p53 autoantibodies (p53-AAb) as detection and prognostic biomarkers in ovarian cancer.

Methods: p53-AAb were detected by ELISA in sera obtained preoperatively from women undergoing surgery for a pelvic mass. This group included women subsequently diagnosed with invasive serous ovarian cancer ($n = 60$), nonserous ovarian cancers ($n = 30$), and women with benign disease ($n = 30$). Age-matched controls were selected from the general population ($n = 120$). Receiver operating characteristic curves were constructed to compare the values of p53-AAb, CA 125, and HE4 as a screening biomarker. Kaplan-Meier curves and Cox proportional hazards modeling were used to assess its prognostic value on survival.

Results: p53-AAb were detected in 25 of 60 (41.7%) of serous cases, 4 of 30 (13.3%) nonserous cases, 3 of 30 (10%) benign disease cases, and 10 of 120 (8.3%) controls (combined $P = 0.0002$). p53-AAb did not significantly improve the detection of cases [area under the curve (AUC), 0.69] or the discrimination of benign versus malignant disease (AUC, 0.64) compared with CA 125 (AUC, 0.99) or HE4 (AUC, 0.98). In multivariate analysis among cases, p53-AAb correlated only with a family history of breast cancer ($P = 0.01$). Detectable p53 antibodies in pretreatment sera were correlated with improved overall survival ($P = 0.04$; hazard ratio, 0.57; 95% confidence interval, 0.33-0.97) in serous ovarian cancer.

Conclusions: Antibodies to p53 are detected in the sera of 42% of patients with advanced serous ovarian cancer.

Impact: Although their utility as a preoperative diagnostic biomarker, beyond CA 125 and HE4, is limited, p53-AAb are prognostic for improved overall survival. *Cancer Epidemiol Biomarkers Prev*; 19(3): 859-68. ©2010 AACR.

Introduction

Changes in tumor antigens, whether due to overexpression, mutation, or altered degradation, can lead to the development of autoantibodies (1). Tumor antigen-specific autoantibodies have been identified in the sera of patients with solid tumors, with antibody levels generally increasing with tumor burden (2-8). The long half-life and *in vitro* stability of these antibodies make them

potential biomarkers for the early detection and/or prognosis of cancer.

As an autoantibody biomarker, p53-AAb are attractive because p53 is mutated in a variety of cancers (9). The development of p53-AAb is associated with tumor p53 mutations that lead to decreased protein degradation (9, 10) and reflect p53-dependent changes in tumor biology. p53-AAb are detected in the sera of 6% to 7% of patients with limited-stage and 19% to 30% of patients with late-stage ovarian cancer (11, 12), suggesting that p53-AAb would have limited application as a diagnostic biomarker. Evidence for the utility of p53-AAb as a prognostic biomarker in ovarian cancer is mixed. Goodell et al. (11) found a correlation with improved overall survival (OS), but two other groups found no correlation with disease-specific survival (12, 13). These differences may reflect patient selection or differences in epitope detection in the assays. It is not known if cancer autoantibodies are involved in active immunologic surveillance, or if they are simply byproducts of altered protein structure found in cancer cells.

We have developed a custom ELISA assay, termed Rapid Antigenic Protein *In situ* Display (RAPID), for the detection of antibodies to tumor antigens in patient

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sera (14). RAPID ELISA eliminates the need for *a priori* protein purification and minimizes the risk of immunogenic copurified bacterial antigens as cross-reacting serologic targets in ELISA. p53-AAb are detected on RAPID ELISA with comparable specificities and limits of detection as standard recombinant protein ELISA for p53 antigen, with a linear range of detection covering three orders of magnitude (8). In this study, we investigated the utility of p53-AAb as biomarkers of diagnosis and prognosis for serous ovarian cancer by itself and compared with the current best ovarian cancer biomarkers, CA 125 and HE4 (15).

Materials and Methods

Patient Sera

Sera used in these analyses were obtained from the Brigham and Women's Hospital and the Dana-Farber Cancer Institute with support from the National Cancer Institute Early Detection Research Network. Sera derived from ovarian cancer patients were obtained at the time of presentation before surgery, and patients received routine postoperative therapy. The nonserous cases were derived from 10 patients with endometrioid cancer, 10 patients with clear cell carcinoma, and 10 patients with mucinous carcinoma. The benign disease samples were derived from 19 patients with serous cystadenomas and 11 patients with mucinous cystadenomas. Sera from age-matched general population control women were obtained from the Brigham and Women's Hospital using a standardized serum collection protocol and were stored at -80°C until use. Cases and matched controls were processed simultaneously. Women with a personal history of cancer (other than nonmelanoma skin cancer) were excluded as controls. Written consent was obtained from all subjects under Institutional Review Board approval. For the 60 serous cases included in the survival analysis, medical records were reviewed and details related to presentation and treatment were abstracted.

RAPID ELISA for p53 Antibodies

The detection of p53 antibodies in patient sera using RAPID ELISA was done as described in ref. (14). Briefly, 96-well detection plates coated with anti-glutathione S-transferase (GST) antibody (GE Healthcare) were blocked overnight at 4°C with blocking buffer (5% milk/0.2% Tween 20 in PBS). On the next day, full-length cDNAs in a pCITE vector optimized for *in vitro* expression (from the Harvard Institute of Proteomics, Cambridge, MA) encoding wild-type p53-GST, p21-GST, EBNA-1-GST, and/or pCITE control vector were expressed using the TNT T7 Coupled Reticulocyte Lysate System (IVTT, Promega) per the manufacturer's recommendations. To each tube of lysate, 16 μL reaction buffer, 8 μL polymerase, 4 μL minus-methionine, 4 μL minus-leucine, 8 μL RNase-OUT (Invitrogen), and 500 ng DNA were added, to a final volume of 400 μL . The DNA-lysate mixture was incubated at 30°C for 90 min. After incubation, PBS was added to the

mixture and 50 μL transferred to each well. The plate was incubated for 2 h at room temperature while shaking at 800 rpm, and then washed five times with blocking buffer. Human serum samples were diluted 1:300 in blocking buffer and 100 μL were added to each well. To test gene expression, mouse anti-GST monoclonal antibody (Cell Signaling Technology) was diluted 1:1,000 in the SuperBlock blocking buffer (Pierce) and 100 μL were added to each well. Secondary antibodies horseradish peroxidase-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch Laboratories, Inc.) or anti-mouse IgG secondary antibody (GE Healthcare) were diluted at 1:1,000. Luminescence detection was done using the SuperSignal ELISA Femto Max Sensitivity (Pierce) and a Wallac plate reader (Perkin-Elmer). Study specimens were tested in duplicate on the same plate.

Detection of CA 125 and HE4 in Sera

The CA 125 assay is a double antibody chemiluminescence immunoassay based on two monoclonal antibodies (M 11 and OC 125, Fujirebio Diagnostics). The Roche CA125II test has been standardized against the Enzy-mun-Test CA 125 II method that in turn was standardized against the CA 125 II RIA from Fujirebio Diagnostics. The analytic sensitivity of the test is 0.6 U/mL. The dynamic range of the assay is 0.60 to 5,000 U/mL, and the reportable range extends up to 25,000 U/mL using a 5-fold dilution. Study specimens were tested in singlet. The imprecision (% CV) of the test, based on two concentrations of quality control materials (35.5 and 104.8 U/mL), was 4.8% to 5.4%. The upper 95th percentile cutoff for healthy premenopausal and postmenopausal women is 35 U/mL.

The method used to measure HE4 in serum is a research-only, double monoclonal ELISA (RK) obtained from Fujirebio Diagnostics, Inc. The assay was done according to the manufacturer's recommendations and results are reported as pmol/L based on the manufacturer's calibration. There is no internationally recognized HE4 reference standard available at this time. The lowest calibrator concentration is 29 pmol/L. The dynamic range of the assay is 15 to 900 pmol/L and the reportable range can be extended by dilution with manufacturer-provided diluent. Study specimens were tested in duplicate on separate plates (each plate contains a calibration curve). The imprecision (% CV) of the test, based on two concentrations of manufacturer-supplied quality control materials averaging 47.1 and 390.4 pmol/L, was 3.8% and 3.4% ($n = 36$ assays), respectively. The imprecision (% CV) of the test determined by measurement of an in-house serum pool averaging 32.4 pmol/L was 8.7% ($n = 36$ assays).

Immunohistochemistry for p53 and Tumor Necrosis

A monoclonal antibody to p53 was used to localize the p53 protein (OP43, Oncogene Science), as previously described (16). A positive score required strong immunostaining obscuring nuclear detail. The percentage of cells staining positive was estimated and recorded for each case.

Statistical Analysis

RAPID ELISA analyses were done in duplicate and average values were used in analyses. Differences between cases and controls were assessed by χ^2 tests. To assess the added value of p53 antibodies along with CA 125 and HE4 to discriminate cases from benign disease and healthy controls, we constructed receiver operating characteristic (ROC) curves and calculated areas under the curve (AUC). Associations between clinical characteristics and p53 antibody detection among cases were tested using logistic regression adjusted for age and Jewish ethnicity. OS was determined from the date of surgery to the date of death or the date last seen in the medical record. We used Kaplan-Meier plots and Cox proportional hazards models to test the associations between marker levels and mortality. All *P* values were two sided. Statistical analyses were done using the SPSS 14.0 software (SPSS, Inc.) and SAS (SAS Institute, Inc.).

Results

Detection of High-Titer p53-Specific Autoantibodies by RAPID ELISA

Antibodies to wild-type p53-GST fusion proteins are detected in the sera of a subset of breast cancer patients by RAPID ELISA (8). In ovarian cancer, p53 antigen is specifically detected by RAPID ELISA at serum titers ranging from 1:600 to 1:48,600, compared with antibodies to the control antigen EBNA-1-GST and vector control (Supplementary Fig. S1A, control curves superimposed, and data not shown). Serum was used at a dilution of 1:300 for all subsequent ELISA analyses. Interassay coefficients of variation of RAPID ELISA is 3.9% (14). Both p21-GST and p53-GST are readily expressed and detected by immunoblotting with anti-GST monoclonal antibody (Mab) (Supplementary Fig. S1B, left), but only p53-GST is detected with the ovarian cancer patient serum (Supplementary Fig. S1B, right).

Detection of p53 Antibodies in Benign and Malignant Ovarian Disease

Sera were collected preoperatively from women undergoing surgery for a pelvic mass. Control sera and questionnaires were collected from healthy women in the Boston area with no history of cancer. The serous cases were primarily stage III/IV (95%). An initial 30 cases of invasive serous cancer and their matched controls were randomly selected as a training set for the detection of p53-AAb by RAPID ELISA, and a cutoff value of 13.1×10^6 was established (mean of the controls + 2 SDs). Using this cutoff, p53-AAb were detected in 13 of 30 cases (43%) and 0 of 30 control sera (*P* < 0.001; Fig. 1A). Table 1 shows the age distribution, menopausal status, and sample collection details of the cases and controls selected for these studies. Cases and controls did not differ in age, race, menopausal status, year of blood collection, or length of storage. When the p53-AAb cutoff value was applied to the entire case and control groups, antibodies were

seen in 25 of 60 (41.7%) of serous cases, 4 of 30 (13.3%) of nonserous cases, 3 of 30 (10%) of benign disease cases, and 10 of 120 (8.3%) of controls (combined *P* = 0.0002; Table 1). We observed a lower frequency of p53-AAb in nonserous ovarian cancer (41.7% in serous carcinoma versus 13.3% in nonserous cases), consistent with a lower frequency of p53 mutations (17). Three of 30 (10%) benign ovarian disease controls were positive for p53-AAb. This is similar to the detection of p53 protein overexpression in 7% of benign ovarian tumors (18). Within the cohort of serous carcinomas, all three cases of early-stage serous carcinoma (one case of stage I and two cases of stage II disease) were positive for p53-AAb.

The distribution of p53-AAb for invasive serous cases (*n* = 60) compared with all controls (*n* = 120) is shown in Fig. 1B and reveals that the distribution is unimodal in controls, with a peak at 9.08×10^6 units. Cases, in contrast, have a bimodal distribution, with a similar baseline peak at 9.08×10^6 units, and a second peak at 30.3×10^6 units. A more stringent cutoff of 16.4×10^6 units (mean normals + 3 SD) increases specificity from 91.7 to 96.7 but decreases sensitivity from 41.7 to 33.3.

Comparison of p53 Antibodies with CA 125 and HE4 Biomarkers

Serum CA 125 and HE4 levels were obtained for the case and controls groups, and are shown in Table 1. Not surprisingly, CA 125 levels were markedly elevated in these (advanced) serous cases versus controls (mean, 775 versus 12; *P* < 0.0001). Similarly, HE4 levels were elevated in serous cases versus controls (mean, 503 versus 61; *P* < 0.0001). Nonserous cases had lower levels of CA 125 (mean, 84), as expected. To determine if p53-AAb have additive value as a screening biomarker for serous carcinoma beyond CA 125 and HE4, ROC curves were constructed comparing all three biomarkers. In this data set, both CA 125 (AUC, 0.99) and HE4 (AUC, 0.98) were superior biomarkers at discriminating cases versus controls, compared with p53-AAb (AUC, 0.69), and p53-AAb added very little to CA 125 and HE4 in combination (Fig. 2A). Nor did we find evidence that p53-AAb (AUC, 0.64) added to CA 125 (AUC, 0.94) or HE4 (AUC, 0.97) in predicting benign disease from invasive serous cases (Fig. 2B).

Detection of p53 Antibodies in the Setting of Low CA 125

In this cohort, CA 125 is elevated in over 95% of cases, due to selection of patients with serous carcinomas undergoing surgery. To determine if p53-AAb have potential additive benefit beyond CA 125 for the detection of serous carcinomas, 20 sera were identified from patients with serous carcinoma who had low CA 125 levels (median, 40; range, 15-76.7). These cases were matched by age and stage with 20 sera with high CA 125 levels (median, 2,116; range, 718-23,010). p53-AAb were detected in 6 of 20 sera (30%) in the low CA 125 cohort, and 11 of 20 (55%) in the high CA 125 cohort. Of the six sera

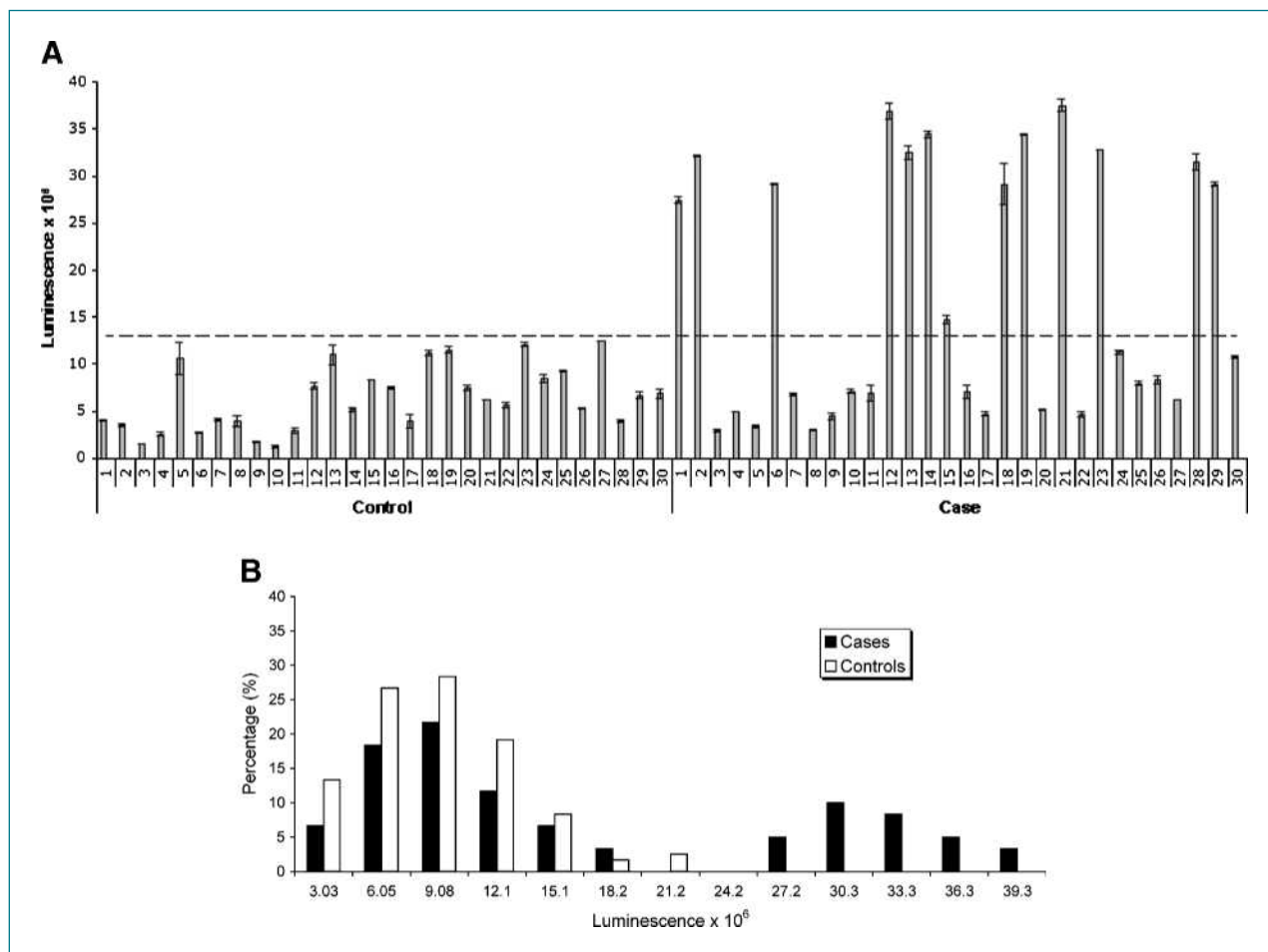


Figure 1. p53 autoantibodies are highly specific biomarkers in serous ovarian cancer. A, sera derived from 30 ovarian cancer patients and 30 age-matched healthy women were tested for p53-specific antibodies by RAPID ELISA. Dotted line, the cutoff value (columns, mean signal of the controls; bars, SD; 13.1×10^6). B, distribution of p53 autoantibodies in serous cases and controls. The signal intensity for serous cases ($n = 60$) and all controls ($n = 120$) are shown as a percentage of the total sera. The distribution of p53-AAb signal intensity for controls (open columns) is a unimodal distribution and the cases show a bimodal distribution (filled columns).

with p53-AAb in the low CA 125 cohort, three had stage I/II, and three had late-stage III/IV serous carcinoma, with a median CA 125 level median of 32.4 (range, 23-76.7).

p53 Antibodies Correlate with Improved Survival in Serous Ovarian Cancer

Table 2 compares the clinical characteristics of serous cases who did not have p53-AAb ($n = 35$) compared with those who had p53-AAb ($n = 25$). No recognized risk factors for ovarian cancer such as pregnancies, oral contraceptive use, or talc use were significantly different between the groups. All patients received standard surgical and adjuvant therapy for advanced serous carcinoma. HE4 and CA 125 levels were not associated with the detection of p53-AAb. There was a tendency for more recent cases to have p53-AAb ($P = 0.05$). However, no significant correlation was found between level of antibodies and length of storage ($r = -0.14$, $P = 0.28$) for se-

rous cases nor for the other groups shown in Table 1 (data not shown). Serous cases with p53-AAb were more likely to have a family history of breast cancer ($P = 0.01$) than those who did not. This may reflect the increased frequency of p53 mutations in the tumors of BRCA mutation carriers (19), although BRCA1 and BRCA2 mutation testing is not available for these cohorts. In eight cases in which we had an assessment of p53 protein overexpression by immunohistochemistry, p53-AAb were detected in two of five cases whose tumors expressed p53 and none of the three cases who were negative by immunohistochemistry, consistent with prior observations that the development of p53-AAb in the sera are strongly correlated with p53 antigen overexpression (9). We saw no difference in the association of p53-AAb and tumor necrosis (data not shown).

We assessed the association between the presence of p53-AAb and survival. To fully adjust this association for other variables related to survival, we conducted a

medical record review of the 60 serous cases and obtained information on clinical and treatment characteristics. All cases were grade 3, and 57 (95%) were stage III/IV. Six patients had residual disease of >1 cm and 36 had ≤1 cm, but residual disease information was unavailable for the remaining 18 cases. The amount of residual disease was highly correlated with stage. Fifty-nine of the 60 cases received chemotherapy, which was adjuvant platinum only with or without paclitaxel known for 39 (66%) patients. Thirty-seven (63%) received at least six cycles of adjuvant chemotherapy. In a model adjusted for age, year of diagnosis, platinum-containing chemotherapy and number of cycles of chemotherapy, stage, and laboratory batch number, we found that p53-AAb were associated with significantly improved survival [hazard ratio (HR), 0.57; 95% confidence interval (95% CI), 0.33-0.97; $P = 0.04$; Table 3]. The association was similar when additionally adjusted for CA 125 and HE4 (data not shown).

Figure 3 illustrates the Kaplan-Meier curves for the two groups. Overall median survival time was 36.8 months

(25th percentile, 22.2 months; 75th percentile, 68.6 months). Thirty-one patients have died (median survival, 28.5 months) and 29 were alive at last follow-up (median survival, 67.2 months).

Discussion

The early diagnosis of ovarian cancer is associated with lower morbidity and longer OS (20), but the majority of ovarian cancers are detected at advanced stages. Serum CA 125 is used as a biomarker for screening high-risk women, but only 50% of early-stage ovarian cancer is detected (21, 22). There remains an urgent need for biomarkers for early detection of ovarian cancers, as well as prognostic biomarkers to help guide therapeutic decisions. Several antigens, including MMP1 (23), cytokines (24, 25), plasminogen activator receptor (26), osteopontin (6), MUC16, MMP7 (27), B7-H4 (28), HE4, and kallikreins (28, 29), have been examined as potential biomarkers for the early detection and prognosis of ovarian

Table 1. Characteristics of cases and controls

	Serous cases (<i>n</i> = 60), <i>n</i> (%)	Nonserous cases (<i>n</i> = 30), <i>n</i> (%)	Benign disease (<i>n</i> = 30), <i>n</i> (%)	Controls (<i>n</i> = 120), <i>n</i> (%)	<i>P</i>
Age					
42-56	20 (33.3)	24 (80.0)	17 (56.7)	62 (51.7)	
57-63	17 (28.3)	3 (10.0)	8 (26.7)	27 (22.5)	
63-71	23 (38.3)	3 (10.0)	5 (16.7)	31 (25.8)	0.99*
Menopausal status					
Pre	8 (13.3)	14 (48.3)	10 (33.3)	40 (33.3)	
Post	52 (86.7)	15 (51.7)	20 (66.7)	80 (67.7)	0.28*
Race					
White	58 (98.3)	25 (86.2)	27 (90.0)	118 (98.3)	
Nonwhite	1 (1.7)	4 (13.8)	3 (10.0)	2 (1.7)	0.05*
Year of specimen collection					
2001-2002	16 (26.7)	8 (26.7)	10 (33.3)	34 (28.6)	
2003-2004	24 (40.0)	12 (40.0)	7 (23.3)	41 (34.4)	
2005-2008	20 (33.3)	10 (33.3)	13 (43.3)	44 (37.0)	0.97*
Length of storage					
<4 y	20 (33.3)	10 (33.3)	13 (43.3)	44 (36.7)	
4-5.7 y	24 (40.0)	12 (40.0)	7 (23.3)	36 (30.0)	
>5.7 y	16 (26.7)	8 (26.7)	10 (33.3)	40 (33.3)	0.57*
p53 status					
Negative	35 (58.3)	26 (86.7)	27 (90.0)	110 (91.7)	
Positive	25 (41.7)	4 (13.3)	3 (10.0)	10 (8.3)	<0.0001 [†]
CA 125 (mean, 95% CI)	775 (500-1,200)	84 (50-140) [‡]	22 (13-35)	12 (11-14)	<0.0001 [§]
HE4 (mean, 95% CI)	503 (379-669)	-	60 (52-69)	61 (58-65)	<0.0001 [§]

* χ^2 test comparing all disease groups to controls.

[†] χ^2 test comparing serous cases to controls. $P = 0.40$ and 0.77 comparing nonserous cases and benign disease subjects to controls, respectively.

[‡]CA 125 values from medical records.

[§] t test comparing serous cases to controls. $P < 0.0001$ and 0.02 comparing nonserous cases and benign disease subjects to controls, respectively. CA 125 and HE4 levels were not measured for the 30 nonserous cases and their matched controls.

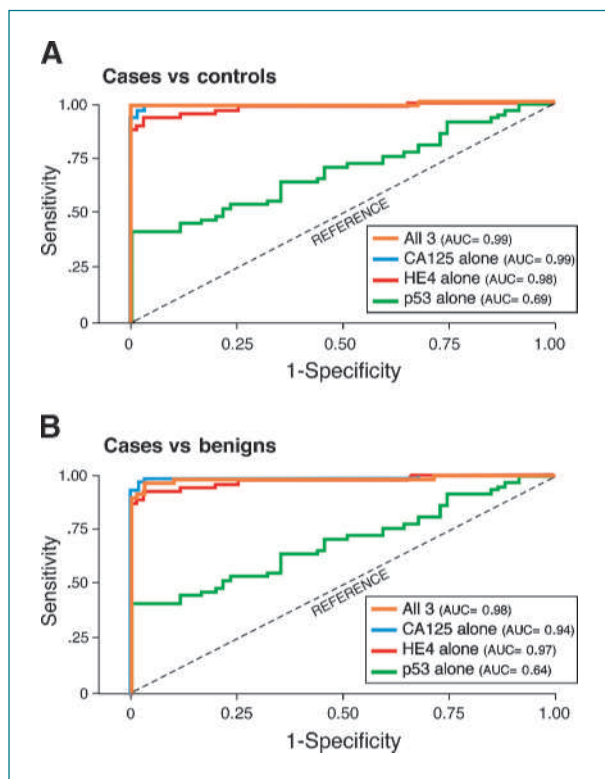


Figure 2. Comparison of ROC curves of p53 autoantibodies, CA 125, and HE4. A, sera derived from 60 serous ovarian cancer patients and 90 age-matched healthy women were tested for CA 125 (blue), HE4 (red), and p53-specific antibodies (green), and ROC curves were calculated. Orange, the combination of all three biomarkers. B, sera derived from 60 serous ovarian cancer patients and 30 women with benign ovarian disease were tested for CA 125 (blue), HE4 (red), and p53-specific antibodies (green), and ROC curves were calculated.

cancer (30). Of these, HE4 has shown promise in addition to CA 125 (15).

p53 Antibodies as Biomarkers for Early Detection

We observed that 41.7% of patients with serous ovarian cancer have p53-specific antibodies. This frequency is higher than published reports of ovarian cancers (16-30% frequency; refs. 11, 12). This is likely due to patient selection, as our results pertain largely to late-stage serous carcinomas, which have a high rate (70-84%) of p53 mutations and overexpression (31). Because this assay uses mammalian IVTT-generated recombinant protein, there may also be differences in antigenic structure between antibody assays. In contrast to serous ovarian cancer, other ovarian cancer subtypes have a lower frequency of p53 mutations, including endometrioid (28%), mucinous (16%), and clear cell carcinomas (10-16%; refs. 17, 19). We confirmed that the frequency of p53-AAb is lower (13.3%) in other ovarian cancer subtypes. No significant differences between cases and controls were observed for endometrioid ($P = 0.39$), mucinous ($P = 0.53$), and clear cell carcinomas ($P = 0.91$; data not shown). Similarly,

we confirmed that p53-AAb are detected at a low frequency in patients with benign ovarian disease (10%). However, we determined that there was limited additive benefit of p53-AAb to the biomarkers CA 125 and HE4 for discrimination of ovarian cancer versus healthy normal women, or of cancer versus benign ovarian disease in this cohort.

Because the majority of specimens in this study were from women with high-grade, advanced invasive serous cancers, the very high frequency of elevated CA 125 and HE4 levels is expected, so that an additive benefit of any diagnostic biomarker in this setting would be minimal. However, p53-AAb were also detected in 30% of patients selected with false-negative CA 125 levels, suggesting that it may still be of benefit as a biomarker for detection and monitoring for this subset of patients.

p53 Antibodies as Biomarkers for Improved Prognosis

Several prior studies have examined the role of p53-AAb as prognostic biomarkers in ovarian cancer (11-13, 32-35). Early studies showed no correlation with OS (33, 34), or association with poor prognosis (36, 37). More recently, in a study of presurgical serum from 104 women with ovarian cancer, p53-AAb were associated with a favorable OS (51 versus 24 months; ref. 11). In contrast, in an extensive study of 233 patients, 15.9% of patients had p53-AAb, which was not associated with any difference in disease-specific survival, even when selected for advanced stage patients (12). A recent study of 130 patients showed an association between p53-AAb and p53 mutation, but not survival (13). Our data agree with the results of Goodell et al. (11) that p53-AAb are associated with a modest favorable prognosis for serous ovarian cancers. We have also shown in multivariate analysis that the detection of p53-AAb is independent of other clinicopathologic risk factors for ovarian cancer, as well as independent of the biomarkers CA 125 and HE4. Although Table 2 suggested the possibility that p53-AAb might be affected by length of storage of the specimen, antibody levels did not correlate significantly with duration of storage. Nevertheless, we included year of diagnosis as an adjustment variable in our model (Table 3), and the association between presence of antibodies and survival remained. We also adjusted for other treatment related variables, such as clinical stage, platinum-containing chemotherapy, and number of cycles of adjuvant chemotherapy, and found that the association between antibodies and survival remained.

The development of p53-AAb is strongly associated with the overexpression of p53 protein and high-grade tumors (10, 12, 35, 38). In two small studies in breast cancer, p53-AAb more commonly developed in the setting of p53 point mutations ($n = 13$; ref. 39) and with mutations that were associated with p53/HSP70 complexes (40). Our data are consistent with the finding that p53-AAb are restricted to patients with tumors that express high levels of p53. The effect of p53 overexpression on prognosis

Table 2. Association between markers, epidemiologic variables, survival, and p53-AAb status among serous cases

	p53 negative (n = 35), n (%)	p53 positive (n = 25), n (%)	P*
CA 125 [mean (95% CI)]	761 (432-1,342)	795 (379-1,667)	0.90 [†]
HE4 [mean (95% CI)]	466 (315-690)	560 (362-868)	0.42 [†]
Year of diagnosis			
2001-2002	12 (34.3)	4 (16.0)	
2003-2004	14 (40.0)	10 (40.0)	0.17
2005-2008	9 (25.7)	11 (44.0)	0.05
Age (y)			
42-56	11 (31.4)	9 (36.0)	
57-63	8 (22.9)	9 (36.0)	0.55
63-71	16 (45.7)	7 (28.0)	0.35
OC use			
Ever	15 (44.1)	15 (60.0)	
Never	19 (55.9)	10 (40.0)	0.15
Parity			
Nulliparous	4 (11.8)	4 (16.0)	
Parous	30 (88.2)	21 (84.0)	0.84
Genital talc use >5 y			
No	26 (76.5)	19 (76.0)	
Yes	8 (23.5)	6 (24.0)	0.92
Jewish ethnicity			
No	31 (88.6)	19 (76.0)	
Yes	4 (11.4)	6 (24.0)	0.40
Smoking			
Never	15 (44.1)	16 (64.0)	
Ever	19 (55.9)	9 (36.0)	0.19
Body mass index			
<20	1 (2.9)	3 (12.5)	
20-24.9	17 (50.0)	9 (37.5)	
25-29.9	9 (26.5)	8 (33.3)	
≥30	7 (20.6)	4 (16.7)	0.77
Tubal ligation			
No	26 (81.2)	21 (84.0)	
Yes	6 (18.8)	4 (16.0)	0.46
Personal history of cancer			
No	31 (91.2)	18 (72.0)	
Yes	3 (8.8)	7 (28.0)	0.10
Family history of breast cancer			
No	31 (96.9)	16 (64.0)	
Yes	1 (3.1)	9 (36.0)	0.01
Immunohistochemistry			
Negative	3 (50.0)	0 (0.0)	
Positive	3 (50.0)	2 (100.0)	0.46
Survival			
Alive	13 (37.1)	17 (68.0)	
Dead	22 (62.9)	8 (32.0)	0.03

Abbreviation: OC, oral contraceptive.

*Adjusted for age, Jewish ethnicity, and batch number.

[†]P value for test of trend.

Table 3. Hazard ratios and 95% CIs for mortality among serous cases

	HR (95% CI)	P
Log-transformed CA 125	1.11 (0.80-1.53)	0.53
Log-transformed HE4	0.81 (0.49-1.34)	0.41
Log-transformed continuous measure for p53	0.56 (0.38-0.93)	0.03
Age, y	1.00 (0.95-1.06)	0.99
Year of diagnosis	1.07 (0.83-1.39)	0.60
	HR (95% CI)	P
Log-transformed continuous measure for p53	0.57 (0.33-0.97)	0.04
Age, y	0.97 (0.92-1.03)	0.36
Year of diagnosis	1.06 (0.80-1.41)	0.67
Adjuvant platinum only with or without paclitaxel	0.81 (0.32-2.07)	0.66
Less than six cycles of first chemo regimen	1.59 (0.44-5.72)	0.48
Unknown number of cycles of first chemo regimen	2.53 (0.81-7.87)	0.11
Stage (<4 vs 4)	2.73 (1.10-6.78)	0.03

NOTE: Data presented here were adjusted for batch number.
Abbreviation: HR, hazard ratio.

is mixed, but has been associated with reduced progression-free survival and OS in ovarian cancer (41-43). In a large study of 783 ovarian carcinomas, p53 overexpression (53%) was associated with shorter OS in univariate analysis, but was only an independent factor for low-intermediate-grade cancers (44). Similarly, in a large multicenter study of 476 patients, p53 expression was not an independent prognostic factor of progression-free survival or OS (45). Our data suggest that p53-AAb identify a cohort of patients with a more favorable prognosis within this high-risk cohort. A prospective study that incorporates full p53 mutational analysis, immunohistochemistry protein expression, and serum p53-Ab detection is needed to confirm these findings.

p53 Antibodies as Biomarkers of Antitumor Immunity

It is not known whether p53-AAb indicate functional immunologic control of tumor, or whether they are surrogate but inert biomarkers for differences in tumor biology. p53-AAb may induce amplification of p53-specific T-cell immunity, which has been detected in tumor-infiltrating lymphocytes in ovarian cancer (46). Because infiltrating T cells are correlated with improved prognosis in ovarian cancer (3), and cell-mediated immunity is associated with improved tumor responses to chemotherapeutic agents (47), it is likely that p53-AAb may help localize cytotoxic T cells to sites of minimal residual disease after chemotherapy.

The development of p53-AAb could also reflect host differences in the induction of antibody responses in general. However, we see no evidence of impaired immune competence to the infectious antigen EBNA-1 in patients who do not make antibodies to p53 (data not shown; ref.

8). Several functional polymorphisms are associated with the enhancement of antibody-dependent cellular cytotoxicity (ADCC) or complement function and autoimmunity. The FCGR2A H131R single nucleotide polymorphism (SNP) (48, 49) regulates Fc binding, and patients who are homozygous for FCGR2A H/H have increased ADCC and rituximab responses (50). Similarly, the CFH Y402H single nucleotide polymorphism regulates complement activation (51), and patients with CFH Y/Y have increased trastuzumab responses (52). In a subset analysis of 16 patients, no association between p53-AAb and the H131R or the CFH Y402H single nucleotide polymorphism was found (data not shown).

Future Applications of p53-Ab Biomarkers

The potential clinical application of p53-AAb as a biomarker for the early detection of ovarian cancer is limited

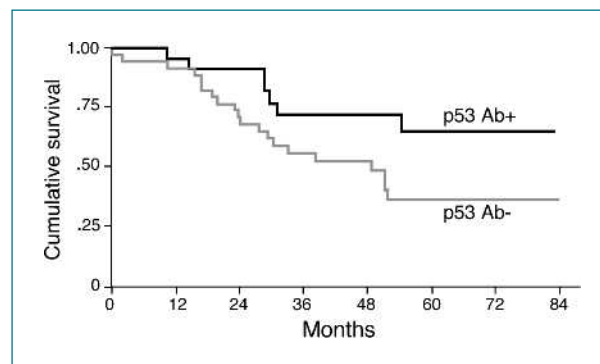


Figure 3. OS is increased in patients with p53 antibodies. Kaplan-Meier curves comparing OS between p53 antibody-positive (black) and antibody-negative (grey) patients.

by the lower sensitivity in early-stage disease (8, 11) and the nonspecific induction of p53-AAb in different p53-overexpressing cancers. However, recent evidence points to early mutations in p53 in the distal fallopian tubes as candidate precursor lesions for ovarian carcinogenesis (16), suggesting that p53-specific biomarkers may still have utility for ovarian cancer diagnosis in the subgroup of BRCA patients who seem to be susceptible to tumors originating in the Fallopian tubes. We did detect p53-AAb in stage I as well as stage II ($n = 3$) serous ovarian cancers, but testing p53-AAb in defined prediagnostic serum collections is required to determine whether p53-AAb have utility prior to clinical diagnosis. To increase both sensitivity and specificity, antibodies to panels of tumor antigens that include p53 have been proposed (3, 4). Recent developments in proteomics technologies, including spotted protein microarrays (5), reversed phase protein arrays (53), phage display (6, 54), and program-

mable protein arrays (8) hold great promise for the future development of autoantibody biomarkers for cancer detection.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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