Application of Bayesian Modeling of Autologous Antibody Responses against Ovarian Tumor-Associated Antigens to Cancer Detection

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Abstract

Biomarkers for early detection of epithelial ovarian cancer (EOC) are urgently needed. Patients can generate antibodies to tumor-associated antigens (TAAs). We tested multiplex detection of antibodies to candidate ovarian TAAs and statistical modeling for discrimination of sera of EOC patients and controls. Binding of serum antibody of women with EOC or healthy controls to candidate TAA-coated microspheres was assayed in parallel. A Bayesian model/variable selection approach using Markov Chain Monte Carlo computations was applied to these data, and serum CA125 values, to determine the best predictive model. The selected model was subjected to area under the receiver-operator curve (AUC) analysis. The best model generated an AUC of 0.86 [95% confidence interval (95% CI), 0.78-0.90] for discrimination between sera of EOC patients and healthy patients using antibody specific to p53, NY-CO-8, and HOXB7. Inclusion of CA125 in the model provided an AUC of 0.89 (95% CI, 0.84-0.92) compared with an AUC of 0.83 (95% CI, 0.81-0.85) using CA125 alone. However, using TAA responses alone, the model discriminated between independent sera of women with nonmalignant gynecologic conditions and those with advanced-stage or early-stage EOC with AUCs of 0.71 (95% CI, 0.67-0.76) and 0.70 (95% CI, 0.48-0.75), respectively. Serum antibody to p53 and HOXB7 is positively associated with EOC, whereas NY-CO-8-specific antibody shows negative association. Bayesian modeling of these TAA-specific serum antibody responses exhibits similar discrimination of patients with early-stage and advanced-stage EOC from women with nonmalignant gynecologic conditions and may be complementary to CA125. (Cancer Res 2006; 66(3): 1792-8)

Introduction

Conventional treatment has limited efficacy against advanced-stage epithelial ovarian cancer (EOC), whereas >80% patients with early-stage disease survive 5 years after diagnosis. However, because there is no diagnostic tool for reliable screening and detection of premalignant or localized ovarian cancer, 70% of patients with ovarian cancer have advanced disease on initial diagnosis (1).

Measurement of serum CA125 levels was approved as a prognostic indicator to monitor disease recurrence (2). Normal healthy donors (~1%) have serum CA125 levels greater than 35 units/mL. Elevated levels of CA125 are detected in >90% of sera of disseminated ovarian cancer cases (stages II-IV) but in only 50% of patients with stage I disease (2). Thus, the CA125 assay is inappropriate as a "stand-alone" population screen for early-stage ovarian cancer, although its positive predictive value can be improved by combination with other screening tools (e.g., serial measurements, transvaginal sonography, or combinations with other markers and statistical modeling).

The immune system constantly surveys the body for "nonself" antigens and generates a response in the appropriate context. Significantly, cancer patients often mount a humoral response to autologous tumor-associated antigens (TAAs; ref. 3). Autologous antibodies have been documented in patients afflicted with a variety of different cancers, including those of the breast, head and neck, colon, lung, kidney, and melanoma (4–6). Ovarian tumor-reactive antibodies have been detected in patient serum and ascites (7) and their antigens are identified by mass spectrometry of immunoprecipitates (8) or by SEREX (refs. 9–11; e.g., ubiquilin-1, ZFP161, FLJ21522, ABC7, HOXA7, and HOXB7). Antibodies to many TAAs are present in several cancer types (e.g., p53 and NY-ESO-1; ref. 12).

Several studies indicate that autologous antibodies specific for TAAs are prevalent in cancer patients but are absent from or infrequent in healthy volunteers. This suggests that autologous antibodies specific to relevant TAAs may have potential as serum biomarkers (13). Therefore, we sought from the literature TAAs associated with ovarian cancer and colon cancer, because the latter often resembles mucinous carcinomas of the ovary. Perhaps the autoantigen best studied in ovarian cancer patients is p53 (14). In stage I/II ovarian disease, 22% of patients had p53 antibody, 31% in stage III, and 50% in stage IV (15). Although there was no association of p53 antibody with clinical stage, tumor histologic type, or overall patient survival (16, 17), detection of autologous

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antibody to some ovarian cancer antigens seems to have prognostic significance (18). Notably, detection of serum antibody to p53 has been shown to predict subsequent development of cancer (19).

The relevance to carcinogenesis of most TAAs is unclear, with the exception of known cell cycle regulators, such as p53, ras, c-myc, c-myb, and HER-2/*neu* (20). The ovarian TAA HOXB7 is overexpressed in ovarian and several other cancers and is associated with enhancement of fibroblast growth factor production and angiogenesis (9, 21). The mechanisms that trigger antibody responses to these autologous TAAs are not known. Although p53 is frequently mutated in cancer, many of these TAAs are not. Many TAAs are overexpressed in the cancer relative to normal tissue or not normally expressed in the tissue, such as the cancer/testis family (22). One study noted that many ovarian TAAs are encoded on chromosome arm 17q (e.g., HER-2/*neu* and HOXB7; ref. 11). Autoantibody to heat shock proteins (Hsp), notably Hsp27 and Hsp90 (23–25), has also been associated with ovarian cancer, and this may reflect the ability of certain Hsps, such as Hsp70, to bind and activate dendritic cells (26).

Although many other TAAs have been identified,[9] the percentage of ovarian cancer patients with reactivity to individual TAAs is generally low. We hypothesized that detection of antibodies to a panel of known TAAs could discriminate sera from ovarian cancer patients and healthy women and potentially improve on the performance of the CA125 assay. However, a statistically rigorous approach to marker selection is required to develop such a clinically applicable diagnostic test by avoiding problems arising from high correlations among potential markers. Herein, we describe the application of multiplex detection of autologous antibodies to a panel of previously described ovarian TAAs and the Bayesian model/variable selection approach using Markov Chain Monte Carlo (MCMC) computations to determine the relevant TAA biomarkers and the most predictive model. MCMC variable selection is a model-based approach with a specified statistical model that puts no distributional restriction on the predictors (markers). Our model-based approach provides probabilistic assessments of uncertainty through Bayesian learning. A unique feature of the Bayesian approach is the easy incorporation of previously described markers in a natural way into existing models, which cannot be achieved by ad hoc procedures, such as recursive partitioning (27–29). Our application of multiplex detection of autologous antibodies to ovarian TAA and Bayesian model selection for detection of EOC complements the CA125 test and implicates p53, HOXB7, and NY-CO-8 in the biology of EOC.

Materials and Methods

Samples. Sera were collected as part of informed consent protocols approved by the local institutional review boards, and the study was approved by the Johns Hopkins University Institutional Review Board. Blood samples were allowed to clot at room temperature and then centrifuged at 400 × *g* to remove clot and cells. Clarified sera were stored at −70°C. A learning set of 59 sera and a validation set of 37 sera were obtained at the University of Louisville School of Medicine (ULSM; Louisville, KY) from women with stage III/IV ovarian cancer and 32 sera from women attending a gynecology clinic for conditions other than ovarian cancer as controls. Sera were also obtained at the University of Texas M. D. Anderson Cancer Center (MDACC; Houston, TX). This set was obtained from women with breast cancer (n = 18), colon cancer (n = 6), lung cancer (n = 10), and stage III/IV ovarian cancer (n = 27) and from healthy women (n = 23). Finally, a set of sera of women with early-stage ovarian cancer (n = 14) was provided by the Gynecologic Oncology Group (GOG; Bethesda, MD).

Preparation of recombinant TAA. TAAs were cloned from PCR products into the prokaryotic expression vector pBADgIII (Invitrogen, Carlsbad, CA). ABC7 (AF133659), HOXA7 (AF032095; ref. 10), HOXB7 (NM_004502; ref. 9), NY-ESO-1 (U87459; ref. 30), ubiquilin-1 (NM_013438), ZFP161 (Y12726), FLJ21522 (AK025175; ref. 11), calmodulin (Invitrogen), and p53 (X02469; ref. 14) were amplified from published constructs, whereas NY-CO-8 (AF039690) and NY-CO-16 (AF039694; ref. 6) were amplified directly from commercial cDNA libraries. Constructs were validated using Automated Laser Fluorescent Sequencing. Bacterial cultures were grown in Terrific broth supplemented with 1% (v/v) glycerol and 100 μg/mL ampicillin at 37°C to mid-log phase (A_{650}, 0.6-0.7) and induced with 0.02% (w/v) L-(+)-arabinose for 2 to 3 hours. Induction was done at 30°C with 0.002% (w/v) L-(+)-arabinose for HOXA7 and HOXB7. Cell pellets from 1 L cultures were solubilized by sonication in 20 mL of 8 mol/L urea, 3.7 mL of 10% (w/v) sodium *N*-lauroyl-sarcosinate and brought to 50 mL with 20 mmol/L Tris-HCl (pH 8.0)/0.2 mol/L NaCl/10% (v/v) glycerol and 0.1% (w/v) sodium *N*-lauroyl-sarcosinate. After centrifugation at 12,000 × *g* for 30 minutes at 4°C, the supernatant was loaded onto a Ni-NTA Superflow (Qiagen, Valencia, CA) column. The column was washed with a step gradient of 10, 20, 50, 100, and 0.5 mol/L imidazole in 20 mmol/L Tris-HCl (pH 8.0)/0.2 mol/L NaCl/10% (v/v) glycerol and 0.1% (v/v) Triton X-100. The purity and size of the purified proteins was determined by staining SDS-PAGE gels for total protein (Sypro Ruby) and performing Western blotting on duplicate gels for His_6-labeled antigen using horseradish peroxidase–labeled anti-His_6 and chemiluminescent substrate.

Coupling to microspheres and Luminex analysis. Monoclonal antibody to His_6 was coupled to 11 distinct sets of LabMAP carboxylated microspheres (following the manufacturer's protocol),[10] which were then individually bound overnight at 4°C with 30 μg each purified His_6-tagged recombinant TAA. Similarly, a further six distinct sets of LabMAP carboxylated microspheres were directly coupled to 25 μg purified Hsp27, Hsp70, and Hsp90 (Stressgen, Victoria, British Columbia, Canada; refs. 25, 31, 32) or, as controls, 25 μg glutathione *S*-transferase (GST) or pBAD vector alone, 5 μg/mL anti-human IgG (Sigma Chemical Co., St. Louis, MO), or 50 μg/mL human IgG. Equivalent counts of each set of protein-coupled microspheres were mixed to a concentration of 5,000 per set per 50 μL/well in PBS containing 10% normal mouse serum (The Jackson Laboratory, West Grove, PA). The beads were shaken with 50 μL patient serum diluted 1:25 in a 96-well filter-bottomed microtiter plate for 1 hour in the dark at ambient temperature. The beads were washed thrice with 100 μL buffer by filtration and then shaken in 100 μL/well R-phycoerythrin (PE)–conjugated donkey anti-human IgG diluted 1:200 in PBS/bovine serum albumin [BSA; 10 g/L BSA, 1.4 g/L $NaH_2PO_4 \cdot H_2O$, 8.77 g/L NaCl, 0.5 g/L NaN_3 (pH 7.4)] for 45 minutes in the dark. After three washes, the beads were resuspended in 100 μL/well PBS/BSA and their mean fluorescence intensity (MFI) was assayed on a Luminex 100 plate reader. The MFI of 100 of each set of microspheres was determined for each well. A small panel of 10 patient sera was run on all assay plates to allow an assessment of interassay variability and bead variability or stability. Several presumptive positive and negative control antigens were included within the bead set, including human IgG (HsIgG to monitor the addition of PE-conjugated secondary antibody), anti-human IgG (αHsIgG to show addition of the human sera to each well), calmodulin (a presumptive irrelevant autoantigen associated with viral hepatitis), or vector alone (controlling for bacterial contaminants in the antigen preparations) and uncoated beads (for assessment of nonspecific binding).

[9] http://www.licr.org/SEREX.html.

[10] http://www.luminexcorp.com.

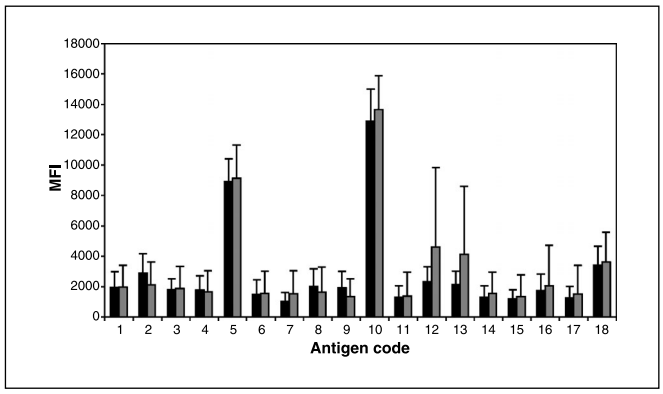

Figure 1. MFI of the beads coated with individual markers (mean ± SD) in normal volunteers (*black columns*) and ovarian cancer patients (*gray columns*). Antigen codes: *1,* FLJ21522; *2,* NY-CO-8; *3,* NY-CO-16; *4,* ABC7; *5,* αHsIgG; *6,* calmodulin; *7,* HOXB7; *8,* HSP70; *9,* HSP90; *10,* HsIgG; *11,* no antigen; *12,* NY-ESO-1; *13,* p53; *14,* ubiquilin-1; *15,* GST; *16,* ZFP161; *17,* HOXA7; *18,* HSP27.

Statistical analyses. A Bayesian logistic regression model/variable selection approach using MCMC (33) computations was implemented in the WinBUGS (34) programming environment. A full description of the model selection and details of our Bayesian computations using Gibbs sampling are provided in the Supplementary Materials.

The motivation behind the variable selection approach was the fact that all the markers and controls were highly correlated (data not shown), which is known as multicollinearity and likely reflects background effects of using serum at high concentration. Thus, simultaneous use of all the markers and controls in a statistical model will obscure the statistical significance of the core variables that are functions of the others. Consequently, the information contained in all the variables is already represented in the core variables, and a dimension/variable selection technique can be use to identify them. In preliminary analyses (data not shown), we ran a logistic regression model that contained all the 13 TAAs and 5 controls and found out that none of the markers and controls were significant, which resulted in poor discrimination of EOC.

The MCMC variable selection approach is a stochastic search algorithm that effectively visits all 2^{18} = 216,144 different models obtained from including/excluding any of the 18 TAAs or controls in a logistic regression for the probability of ovarian cancer. The outcome of this procedure is the model with the highest number of visits (the most probable model) supported by the data. For numerical stability, we transformed the measured MFI levels of the markers and controls to logarithmic scale. Our approach adjusts for the associations among the 13 markers and the effects of the 5 controls. Given the limited number of cases and controls, we focused on an additive model assuming no interactions among the markers, controls, and between markers and controls. *A priori*, each specific marker or control is assumed to be equally likely to be included/excluded (i.e., with probability of 0.5) in the model. This corresponds to an equally likely prior probability of $1/2^{18}$ for each possible configuration in the model space. The inclusion probability for a specific marker or control is then updated by their posterior probability using all the available information about the other markers and controls, conditional on the observed ovarian cancer status. Although, in principle, one can compute the posterior probability of each of the 2^{18} models using the Bayesian analysis, a simpler alternative is to use the Bayes factor (BF; ref. 35), defined as the posterior and prior odds ratio, to select relevant markers or controls for future analyses. For a specific marker or control, larger values of BF provide evidence in favor of inclusion, whereas smaller values support exclusion from the model. Usually, a value between 1 and 3 is considered weak, between 3 and 10 as substantial, between 10 and 100 as strong, and >100 as very strong (36).

Results

To explore the utility of autologous serum antibody specific to particular TAAs as an additional tool for discrimination of sera from cancer patients and healthy women, we generated recombinant protein from 13 markers identified previously as candidate TAAs and selected 5 presumptive control reagents. Sera from both healthy women and EOC patients were tested in parallel for reactivity to all 18 panel elements. Initially, we tested sera of 23 healthy women from MDACC as controls and sera from 59 stage III/IV ovarian cancer patients obtained at ULSM and 27 from MDACC as cases, respectively. This set of sera was analyzed for reactivity to microspheres of discrete sizes coated with individual recombinant TAA or control protein. The presence of antibody bound to the beads was detected with PE-labeled anti-human IgG and the fluorescence was quantified using a Luminex plate reader. Because 5 presumptive control elements were included in the panel, absolute MFI values without background subtraction were employed (Fig. 1).

Our analytic strategy consisted of fitting the logistic regression model in Eq. 1 (see Supplementary Materials) for ULSM and MDACC cases and controls. By using BF $\geq$ 3 as a selection

Table 1. Modeling of serum responses to the 18-element panel in cases and controls

Marker	$\Pr(d_j = 1 \mid Data)$	θ_j	BF_j
FLJ21522	0.49 (0.50)		
NY-CO-8	0.98 (0.15)	−1.49 (0.62)	49
NY-CO-16	0.45 (0.49)		
ABC7	0.65 (0.48)		
αHsIgG	0.42 (0.49)		
Calmodulin	0.42 (0.49)		
HOXB7	0.92 (0.27)	1.42 (0.76)	11.5
Hsp70	0.57 (0.49)		
Hsp90	0.49 (0.50)		
HsIgG	0.43 (0.49)		
No antigen	0.60 (0.49)		
NY-ESO-1	0.48 (0.50)		
p53	0.96 (0.20)	1.21 (0.58)	24
Ubiquilin-1	0.61 (0.49)		
ZFP161	0.28 (0.45)		
Vector	0.40 (0.49)		
HOXA7	0.42 (0.49)		
Hsp27	0.42 (0.49)		
	Post. mean (SD)	95% CI for AUC	
AUC	0.86 (0.03)	0.78	0.90

NOTE: Identification of the best-fitting model for discrimination between sera of 23 healthy patients from MDACC as controls and sera from 59 stage III/IV ovarian cancer patients obtained at ULSM and 27 from MDACC as cases using the 18-element panel. Data are posterior estimates (mean and SD) of $\Pr(d_j = 1 \mid Data)$ for all the variables, the regression coefficients $\{\theta_j = d_j b_j\}$, and BFs for the best markers and controls (selected by the Bayesian approach) using the data set of ULSM and MDACC cases and controls. The regression coefficients θ_j are assumed to be statistically independent, and each have a two-component mixture prior distribution with a point mass at 0 (when $d_j = 0$) with probability of 0.5 and a normal distribution $N(0, \sigma_j^2)$ (when $d_j = 1$) with probability 0.

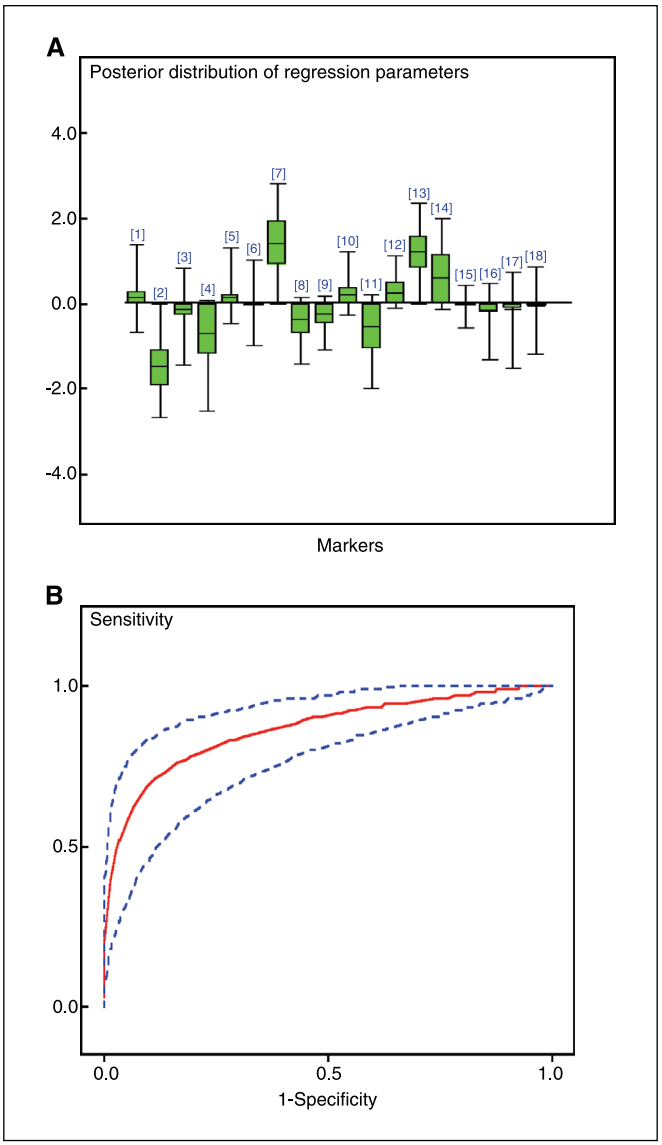

Figure 2. Posterior distributions and estimate of ROC for markers NY-CO-8, HOXB7, and p53. *A,* posterior distributions of $\{\theta_j\}$ in the variable selection algorithm for data of Table 1. *Numbers inside brackets,* elements in Table 1 (i.e., *[1]* corresponds to FLJ21522, etc.). *B,* posterior estimate of ROC for markers NY-CO-8, HOXB7, and p53 with 95% credible interval for cutoff values ranging from 0.01 to 1.00 for data of Table 1.

criterion, we picked the best model and computed multiple sensitivity, specificity, ROC, and area under the receiver-operator curve (AUC) by varying a probability cutoff between 0.01 and 1.00 (Table 1; Fig. 2). The AUC was calculated as 0.86 [95% credible interval (Bayesian analogue of confidence interval), 0.78-0.90] for this best-fitting model (Table 1). The best model used three TAA markers: p53 and HOXB7, which showed a positive association, and NY-CO-8, which showed a negative association (Table 1).

We also compared the best model for discrimination of cases and controls with detection of absolute serum level of CA125 or p53-specific antibody or both. The AUC was calculated as 0.83 [95% confidence interval (95% CI), 0.81-0.85] for the standard CA125 assay alone. Detection of p53 antibody alone was not useful for discrimination of cases and controls in this serum set (AUC, 0.52; 95% CI, 0.20-0.58) and combination of absolute CA125 level and serum reactivity to p53 provided an AUC of 0.81 (95% CI, 0.79-0.83). We tested whether addition of absolute serum level of CA125 as an element of the panel conferred additional predictive value on reanalysis of the serum set (Table 2). The posterior distributions of the regression variables are displayed in Fig. 3*A* and the ROC is given in Fig. 3*B* (along with 95% credible bands). Although there is improvement in terms of the accuracy of the fitted model [AUC with CA125 goes slightly up from 0.86 (95% CI, 0.78-0.90) to 0.89 (95% CI, 0.84-0.92)], the same markers NY-CO-8, HOXB7, and p53 are again selected as most informative along with CA125.

We conducted a cross-validation analysis to determine the self-consistency of the best model. Here, a leave-one-out approach was used where each data point D_i was removed from the data set and predicted (using the best-fitting model in Table 1 and posterior predictive distribution $[D^*_i|D_{\{-i\}}]$) by the remaining cases $D_{\{-i\}}, i = 1, 2, \ldots, n$ with $\{-i\}$ denoting the data, except for the ith observation and D^*_i is a predicted value of D_i. The resulting cross-validated ROC in Fig. 4*A* was very close in shape to ROC given in Fig. 2*B*. The posterior mean of the cross-validated AUC was 0.85, with a slightly wider 95% credible interval of 0.76 to 0.92 reflecting additional uncertainty arising from predictions. Therefore, for this data set, our model is self-consistent.

Several conditions unrelated to ovarian cancer result in an elevated serum CA125 level. Furthermore, the sensitivity of the CA125 for detection of early-stage disease is limited. To validate

Table 2. Modeling of the serum responses to the 18-element panel and CA125 level in cases and controls

Marker	$\Pr(d_j = 1\|Data)$	θ_j	BF_j
FLJ21522	0.37 (0.48)		
NY-CO-8	0.90 (0.31)	−1.18 (0.67)	9
NY-CO-16	0.44 (0.50)		
ABC7	0.53 (0.50)		
αHsIgG	0.35 (0.48)		
Calmodulin	0.42 (0.49)		
HOXB7	0.87 (0.33)	1.17 (0.73)	6.7
Hsp70	0.33 (0.47)		
Hsp90	0.41 (0.49)		
HsIgG	0.36 (0.48)		
No Antigen	0.52 (0.50)		
NY-ESO-1	0.41 (0.50)		
p53	0.76 (0.43)	0.73 (0.62)	3.2
Ubiquilin-1	0.52 (0.50)		
ZFP161	0.49 (0.50)		
Vector	0.41 (0.50)		
HOXA7	0.43 (0.49)		
Hsp27	0.37 (0.48)		
CA125	0.99 (0.07)	0.90 (0.37)	99
	Post. mean (SD)	95% CI for AUC	
AUC	0.89 (0.03)	0.84	0.92

NOTE: Identification of the best-fitting model for discrimination between sera of 23 healthy patients from MDACC as controls and sera from 59 stage III/IV ovarian cancer patients obtained at ULSM and 27 from MDACC as cases using the 18-element panel and absolute CA125 level. Statistical analysis was done as described for Table 1.

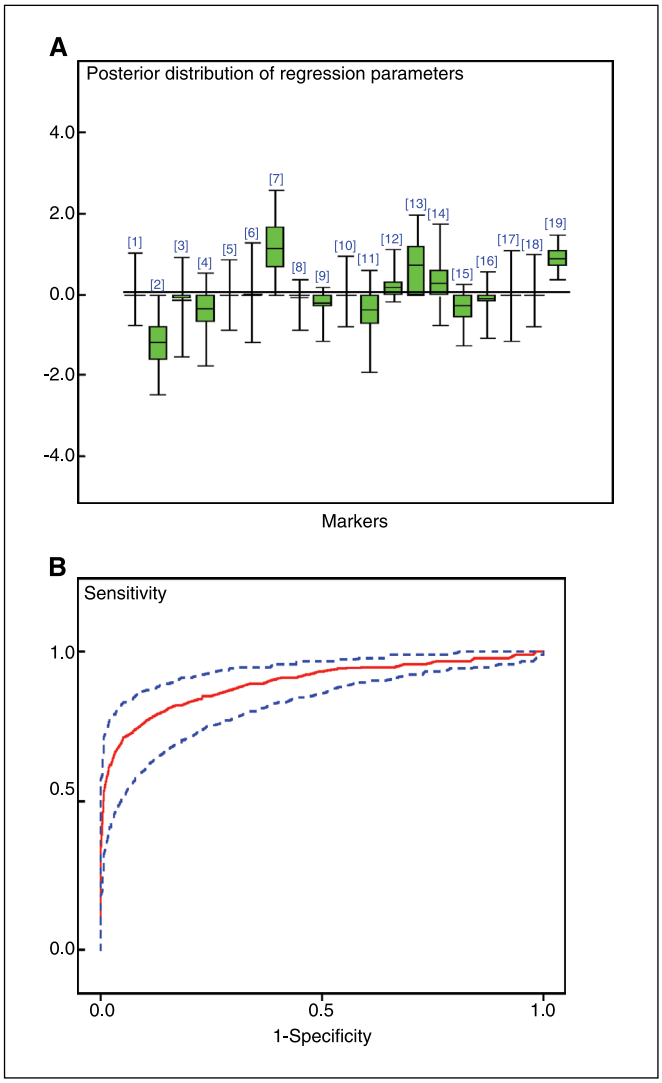

Figure 3. Posterior distributions and estimate of ROC for markers NY-CO-8, HOXB7, p53, and CA125. *A,* posterior distributions of $\{\theta_j\}$ for all 18 candidate markers plus CA125 levels (*[19]*) in the variable selection algorithm for data of Table 2. *B,* posterior estimate of ROC for training set using NY-CO-8, HOXB7, p53, and CA125 with 95% credible interval for cutoff values ranging from 0.01 to 1.00 for data of Table 2.

our 18-element panel and modeling in a serum set in which the CA125 assay would perform poorly, we studied sera of 32 women attending a gynecology clinic at ULSM (including 3 with CA125 levels >35 units/mL) but who had no evidence of ovarian cancer as controls. As cases, we tested sera from either 37 women with advanced-stage (III/IV) ovarian cancer or 14 women with early-stage (I/II) ovarian cancer (Fig. 4*B* and *C*). These test sets of independent sera were analyzed in parallel for reactivity to the panel of TAAs and controls, and the data were analyzed using the previously determined best-fitting model excluding CA125 (described in Table 1). The ROC analyses are provided in Fig. 4*B* and *C*. The model provided an AUC of 0.71 (95% CI, 0.67-0.76) for discrimination between women with advanced-stage ovarian cancer and those attending the gynecology clinic at ULSM and an AUC of 0.70 (95% CI, 0.48-0.75) for discrimination between early-stage ovarian cancer and women attending the gynecology clinic at ULSM.

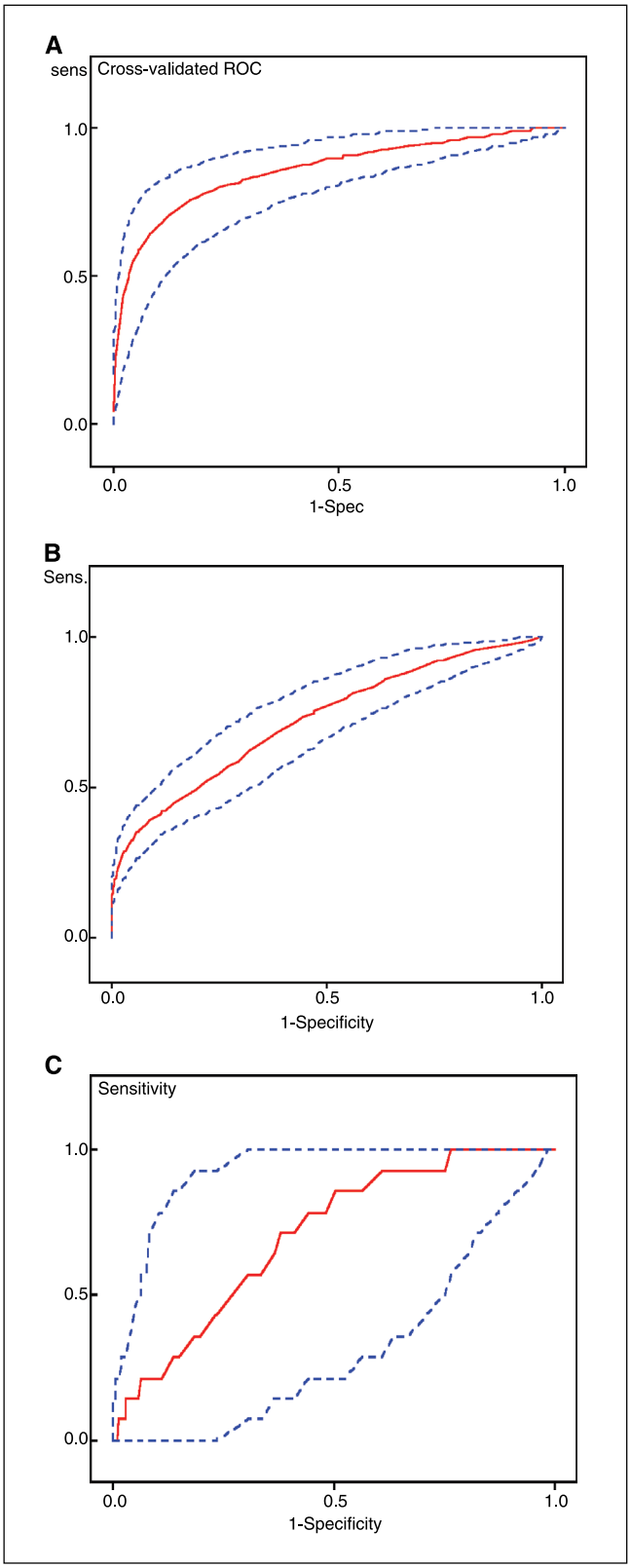

Figure 4. Validation steps. *A,* cross-validated estimate of ROC for the best-fitting model summarized in Table 1. *B* and *C,* cross-validation of the best-fitting model for p53-, NY-CO-8-, and HOXB7-specific antibody reactivity (see Table 1) using an independent set of sera from 37 advanced-stage ovarian cancer patients (*B*) or 14 early-stage ovarian cancer patients (*C*) as cases and 32 women attending a gynecology clinic for conditions other than ovarian cancer as controls in (*B* and *C*).

Discussion

Our study examines the applicability of multiplex detection of autologous antibody to TAAs as a diagnostic tool for cancer. The detection of autologous antibody to this three-member panel of TAAs, in addition to CA125, shows merit as an approach for ovarian cancer detection. However, much larger studies are required to assess its sensitivity at the high specificity required for screening healthy women. In particular, it warrants further examination in high-risk populations, such as female first-degree relatives of ovarian cancer patients or women carrying mutations that predispose toward cancer. The application of longitudinal screening may also significantly increase the positive predictive value of the test (37, 38). Sera collected over up to four time points from individual patients with ovarian (n = 5) or breast (n = 5) cancer were analyzed for the stability of TAA reactivity. The reactivity to individual TAAs was generally stable over several years despite therapy, indicating that this approach is not useful to monitor therapy (data not shown).

Our modeling of TAA-specific antibody discriminates poorly the patients with ovarian cancer from those with cancer of other organ sites, notably women with breast cancer (n = 18), colon cancer (n = 6), and lung cancer (n = 10; data not shown). This reflects the use of p53 as a diagnostic marker and its importance in cancers in addition to ovarian cancer (14). However, other investigators have improved specificity for a particular cancer type by the inclusion of additional markers (29, 39). Over 2,000 TAAs have been entered into the SEREX database.[9] Several other approaches have also been used to identify candidate TAAs, including mass spectrometric analysis of immunoprecipitates (8) and screening of phage display libraries (40, 41). Inclusion of other TAAs using similar, highly multiplexed approaches (42) in large case-control studies may provide better discrimination between ovarian and other cancer types.

A more conventional approach to data analysis using logistic regression where all the 13 markers and 5 controls were forced to stay in the model (with prior probability of inclusion being 100% for each) failed to provide a useful model (data not shown), as an AUC of only $\sim$0.60 was obtained. Here, we describe the application of Bayesian model/variable selection approach using MCMC computations, implemented in the freely available WinBUGS program, to determine the best predictive model. The MCMC variable selection is a model-based approach with a specified statistical model on the cases and controls (in our case, binomial distribution with logistic link) but puts no distributional restriction on the predictors (markers). It represents an alternative to the existing ad hoc statistical approaches, such as recursive partitioning (27–29), and provides formal assessment of uncertainty (in probabilistic terms) through Bayesian learning and updating. A unique feature of our methods is the easy incorporation in a natural way into existing models of prior information obtained from earlier clinical studies or scientist's experience that cannot be used in other procedures.

We conducted a cross-validation analysis initially using a leave-one-out approach to determine the self-consistency of our best model for discrimination between healthy volunteers and women with advanced-stage ovarian cancer. The model has proven to be internally consistent; therefore, we sought further validation using an independent serum set. Unlike advanced-stage disease, ovarian cancer diagnosed while still confined to the ovaries is highly curable using current interventions. Thus, we examined the ability of our model to discriminate sera from patients with advanced-stage and early-stage disease. The model was tested in another set of sera that included 14 from patients with stage I/II ovarian cancer or 37 sera from advanced-stage ovarian cancer patients. Furthermore, as a more rigorous test, we used sera from women with nonmalignant gynecologic conditions as controls. The model achieved an AUC of 0.70 (95% CI, 0.48-0.75) for discrimination between sera of early-stage ovarian cancer patients and controls. The wider credible intervals partially reflect the relatively small number of early-stage cases (n = 14). This outcome represents a significantly poorer performance than for advanced-stage cases versus healthy volunteers for which an AUC of 0.86 (95% CI, 0.78-0.90) was derived. However, we obtained an AUC of 0.71 (95% CI, 0.67-0.76) for discrimination between advanced-stage ovarian cancer and women with nonmalignant gynecologic conditions suggesting that these markers, like CA125, are affected by nonmalignant gynecologic conditions.

Many other biomarkers, including CA125, are significantly less predictive for early-stage ovarian cancer patients. It is therefore interesting that the autoantibody-based test may be similarly effective for discrimination of sera of both early-stage and late-stage ovarian cancer patients from healthy women. Although no information is available on the relationship of NY-CO-8 and ovarian carcinogenesis, both p53 mutation (14, 16, 43) and HOXB7 overexpression have been described in early-stage ovarian cancer (11, 21).

Differences in specimen processing can confound biomarker studies. The similar AUCs obtained using ovarian cancer cases from different institutions (ULSM and GOG) suggests that this autoantibody-based test is likely resistant to the confounding effects of collection at different sites.

Pattern recognition analysis of proteomic profiles has been used to discriminate sera of ovarian cancer patients from those of healthy women. However, the biological underpinning of these patterns is yet unclear (44). By contrast to proteomic profiles, the significance of the TAAs p53 and HOXB7 in cancer biology has been studied extensively for ovarian and other cancers (9, 43, 45–47). A partial cDNA sequence NY-CO-8 was initially identified as a colon cancer antigen. However, subsequent analysis of the serologic reactivity to NY-CO-8 suggests that it is a naturally occurring autoantigen (42). The presence of NY-CO-8-reactive autoantibody in healthy controls that are suppressed in cancer patients may account for its negative association with ovarian cancer in our study. However, we observed no generalized suppression of antibody levels in ovarian cancer patients (Fig. 1); thus, the possibility of a protective effect warrants further investigation. Indeed, a similar phenomenon was recently described for MUC1-specific antibody (48). The full-length NY-CO-8 gene was recently cloned, and its product, CCCAP, was shown to associate with centrosomes (49). Intriguingly, disruption of the centrosomes is an early event in the genesis of ovarian cancer. Furthermore, the breast and ovarian cancer hereditary susceptibility genes BRCA1 and BRCA2 contribute to a centrosome function that is believed to help maintain the integrity of the chromosome segregation process (50, 51).

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