Soluble Epidermal Growth Factor Receptor (SEG-FR) and Cancer Antigen 125 (CA125) as Screening and Diagnostic Tests for Epithelial Ovarian Cancer

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Abstract

Epithelial ovarian cancer (EOC) is the leading cause of death among all gynecologic cancers in the United States. Because women who are diagnosed with early stage disease have a better prognosis than women diagnosed with late stage disease, early detection represents a potentially practical approach to reduce the mortality associated with EOC. Unfortunately, no single screening test has proven to be effective for this purpose, and a valid and feasible screening program to detect early stage EOC in the general population has not yet been devised. Consequently, research has focused on coupling two or more screening modalities to improve program validity and feasibility. Serum cancer antigen 125 (CA125) and a soluble isoform of the epidermal growth factor receptor (p110 sEGFR) have been studied individually as biomarkers of ovarian cancer. In this study, we compare serum CA125 levels and sEGFR concentrations in women with EOC to women with benign gynecologic conditions of ovarian and non-ovarian origin. We show that serum sEGFR concentrations are lower in patients with EOC than in women with benign gynecologic conditions, whereas serum CA125 levels are higher in patients to EOC compared with women with benign gynecologic conditions. These data also reveal that age and serum sEGFR concentrations modify the association between CA125 levels and EOC versus benign gynecologic disease. Hence, age- and sEGFR-dependent CA125 cutoff thresholds improve the ability of CA125 to discern EOC patients from women with benign ovarian tumors and non-ovarian gynecologic conditions. Our analyses show that parallel testing with fixed sEGFR and CA125 cutoff thresholds optimizes sensitivity to detect EOC, whereas serial testing with age- and sEGFR-dependent CA125 cutoff thresholds optimizes test specificity, and overall accuracy to discern patients with EOC from women with benign ovarian and non-ovarian gynecologic conditions. The combined use of serologic sEGFR and CA125, thus, has improved utility for screening and diagnosing EOC, which may increase the positive predictive value of a multimodal screening program that incorporates these biomarkers to detect and subsequently differentiate benign from malignant ovarian tumors. (Cancer Epidemiol Biomarkers Prev 2005;14(2):306–18)

Introduction

Epithelial ovarian cancer (EOC), which represents 95% of all ovarian cancer cases (1, 2), affects the lives of thousands of women and their families each year. EOC ranks 5th in the incidence of all cancers in women and remains the leading cause of death among gynecologic malignancies in the United States; 25,580 new cases and 16,090 deaths from this disease were estimated for 2004 (3). Similar incidence and mortality rates for EOC exist in the United Kingdom and Europe (4). Women with EOC often are asymptomatic or have vague, nonspecific symptoms such as indigestion, bloating, and changed bowel habits; hence, late stage diagnosis is the norm (5). Moreover, the high mortality rate of EOC is a consequence of the fact that 70% to 75% of women with EOC are diagnosed with stage III or IV disease, which has 5-year survival rates of just 15% to 31% (3, 6-8). In comparison, 5-year survival rates for stage I EOC patients are significantly better, in the range of 90% to 95% (3, 9, 10). Despite new therapy options (11, 12), the age-adjusted mortality rate for patients with EOC has not changed significantly over the past 20 years (3, 13). Early detection, therefore, is a potentially practical approach for controlling EOC.

Unfortunately, cost-effective, valid, and feasible methods that can be recommended to screen for EOC in the general population do not exist yet (14). Cytologic evaluation of cervical, vaginal, or peritoneal fluids (15-18), pelvic examination (19-21), transabdominal, transvaginal, and color Doppler sonography (22, 23), and numerous biomarkers (24, 25) have been studied as potential screening and diagnostic tests for EOC. However, these modalities are not endowed individually with sufficient test sensitivity and specificity (accuracy) to be recommended for population-based screening of ovarian cancer. Nor are these modalities useful as stand-alone diagnostic tests. Consequently, contemporary research has focused on coupling two or more modalities “in parallel” or “in series” to improve either test sensitivity or specificity, respectively (26-30).

Cancer antigen 125 (CA125), a heterogeneous cell membrane glycoprotein that ranges in molecular weight from 200 to 500 kDa encoded by the MUC16 gene (31, 32), has been studied extensively as a potential screening and diagnostic
test of EOC (33-37). Immunoassay studies have shown that serum CA125 (>35 units/mL cutoff) levels are elevated in 14% of healthy women and 82% of all EOC patients; but only 50% of EOC patients with stage I disease (38-41). Malkasian et al. (42) further showed that CA125 has a greater sensitivity (81% versus 60%) and specificity (91% versus 73%) for distinguishing EOC from benign gynecologic conditions among postmenopausal women than premenopausal women. Others have confirmed this observation (43-45). However, elevated serum CA125 levels are not restricted to EOC. Notably, serum CA125 levels are elevated in various normal and pathologic conditions that affect the endometrium, including menstruation (46-50), pregnancy (51-56), endometriosis (20, 42, 57-61), and endometrial cancer (58, 62, 63). Serum CA125 levels also are elevated in patients with benign and inflammatory diseases of the liver (64, 65), pelvis (66), uterus (58), and ovary (42, 53, 58, 67), and in patients with hematologic, bladder, breast, fallopian, gastrointestinal, liver, lung, and pancreatic cancers (68-70). Despite CA125’s elevation in other diseases, immunoassay studies have documented specificities ranging between 95.4% and 96.7% (40, 42, 71, 72).

Soluble epidermal growth factor receptor (sEGFR/sErbb1) is a 110-kDa glycoprotein found in human serum that is encoded by a 3.0 kb alternate mRNA transcript of the EGF gene (73, 74). Immunoassay studies have shown that patients with EOC have significantly lower serum p110 sEGFR concentrations than healthy women, and that sEGFR concentrations are inversely associated with serum concentrations of follicle-stimulating hormone and luteinizing hormone, as well as with age in healthy women (74, 75). Moreover, age and menopausal status modify the association between sEGFR concentrations and EOC versus healthy women (74). By using menopausal status-dependent cutoff thresholds to maintain 95% test specificity across strata, serum sEGFR concentrations were found to have 74% sensitivity to detect EOC among premenopausal women, but only 50% sensitivity to detect EOC among postmenopausal women. Test sensitivity was lower for detecting stage I/II compared with stage III/IV EOC among premenopausal women (64% versus 81%) and postmenopausal women (28% versus 54%). Stratification of EOC cases and healthy controls into groups between 20 to 40, 41 to 60, and 61 to 87 years of age, followed by selection of age-dependent cutoff values showed a sensitivity of 72.7%, 60.6%, and 33.3% for each age group at 95% specificity, respectively. Likelihood test sensitivity for detecting stage I/II or stage III/IV EOC from healthy women in these age groups was better for late stage versus early stage disease. Thus, serum sEGFR concentrations seem to be most useful for detecting EOC among younger, premenopausal women.

Protein biomarkers present in tissues or biofluids, in theory, may be useful to detect the presence of a tumor (screening) and/or to discern malignant from benign tumors (diagnosis). Here, we evaluate the combined utility of serum CA125 and p110 sEGFR for screening and/or diagnostic application by comparing women with EOC (stages I-IV) to women undergoing surgery for benign ovarian tumors, and benign gynecologic conditions of non-ovarian origin. We report that parallel testing with sEGFR and CA125 optimizes test sensitivity to detect EOC, whereas serial testing with sEGFR-dependent CA125 cutoff thresholds optimizes test specificity, as well as accuracy to discriminate patients with EOC from women with benign ovarian and non-ovarian gynecologic conditions. We conclude that testing with both serologic sEGFR and CA125 improves the ability to detect EOC, as well as to discern EOC from benign gynecologic disease, and, therefore, warrants further investigation for the early detection and subsequent diagnosis of ovarian cancer.

Materials and Methods

Serum Samples. Preoperative serum samples from 225 women with incident EOC were obtained from repositories at the Mayo Clinic, Department of Gynecologic Surgery; the National Cancer Institute, Cooperative Human Tissue Network, Gynecologic Oncology Group Ovarian Tumor Bank; and the National Cancer Institute Ovarian Cancer Early Detection Program at Northwestern University, as previously described (74). Patient age, menopausal status, International Federation of Gynecology and Obstetrics disease stage, tumor histologic subtype, and tumor grade were made available from the Mayo Clinic, the Gynecologic Oncology Group, and the National Cancer Institute Ovarian Cancer Early Detection Program databases. Briefly, 31, 13, 145, and 35 of the EOC cases had stage I, II, III, and IV disease, respectively. The cases included 23 grade I, 36 grade II, and 145 grade III tumors of the following histologic subtypes: 109 papillary serous, 41 serous, 17 endometrioid, 18 primary peritoneal, 14 mixed, 4 clear cell, 3 mucinous, and 1 transitional cell carcinoma. Information concerning stage, grade, and histologic subtype was not available for 1, 21, and 18 EOC cases, respectively.

Serum samples from women scheduled for gynecologic surgery at the Mayo Clinic were collected between 1985 and 1994 to study the reproducibility of CA125 measurements (40, 41, 58, 76). From this repository, we identified preoperative serum samples from 246 patients with benign ovarian neoplasms and 253 patients with benign gynecologic conditions of non-ovarian origin by reviewing the pathology reports. Of the 246 women with benign ovarian tumors, 81 women had a pathologic diagnosis of a functional ovarian neoplasm and 165 women had a pathologic diagnosis of a nonfunctional ovarian neoplasm. Patients with functional ovarian neoplasms had the following types of cysts: 10 follicular, 17 corpus luteum, 20 hemorrhagic corpus luteum, and 34 simple. Patients with nonfunctional ovarian neoplasms included the following types: 41 mature teratoma, 9 fibroma, 31 mucinous cystadenoma, 1 mucinous cystadenofibroma, 39 serous cystadenoma, 43 serous cystadenofibroma, and 1 seromucinous cystadenoma. Of the 253 patients with benign gynecologic conditions of non-ovarian origin, 109 had endometriosis. The remaining 144 patients with non-ovarian gynecologic conditions had the following pathologic diagnoses: 43 cervical dysplasia, 80 uterine leiomyoma, 10 hydrosalpinx, 43 leiomyoma, and 11 paratubal cyst. Patient age and menopausal status at the time of surgery were abstracted from each patient’s medical record. Menopausal status was determined as described previously (76).

sEGFR Acridinium-Linked Immunosorbent Assay. Serum p110 sEGFR concentrations were determined by acridinium-linked immunosorbent assay (ALISA; 75, 77). Initially, all sera were diluted 1:10 in ALISA blocking buffer and assayed in duplicate in three separate trials. Serum samples yielding relative light units below the linear range of the assay’s standard curve were re-assayed either undiluted or diluted 1:5 in ALISA blocking buffer, whereas serum samples yielding relative light units above the linear range of the assay’s standard curve were re-assayed either diluted 1:20 or 1:50 in ALISA blocking buffer. For each trial, the mean relative light unit for each duplicate was determined and a corresponding sEGFR concentration in fmol/mL was calculated. The reported sEGFR concentration given in fmol/mL for each serum sample is the median value from these three trials. The interassay biological detection limit (4.5 SD above the zero calibrator) for the ALISA done in this study was 7.5 fmol/mL sEGFR.

CA125 ELISA. CA125 levels were determined previously using the Centocor II assay for all serum samples obtained from the Mayo Clinic repository (40, 41, 58, 76). These values...
were abstracted from patient medical records. CA125 levels were made available from the Gynecologic Oncology Group and the National Cancer Institute Ovarian Cancer Early Detection Program databases.

**Statistical Analysis.** Descriptive statistics for serum p110 sEGFR concentrations and CA125 levels were calculated for women with EOC, benign ovarian neoplasms, and benign non-ovarian gynecologic conditions using JMP statistical analysis software (SAS Institute, Inc., Cary, NC.). The Wilcoxon rank-sum test was used to determine if significant differences in sEGFR concentrations or CA125 levels exist between these three groups of women, before and after stratification based on menopausal status. Spearman’s rank-order correlation coefficient (p) was used to determine whether associations exist between sEGFR concentrations or CA125 levels and age in women with EOC, benign ovarian neoplasms, and benign gynecologic conditions of non-ovarian origin.

Test specificity, sensitivity, and accuracy were calculated using tabular methods for fixed and variable cutoff thresholds for sEGFR concentrations and CA125 levels to evaluate the usefulness of these biomarkers as individual and combined tests for EOC. The combination of sEGFR and CA125 was evaluated using “in series” and “in parallel” testing algorithms (78). Parallel testing involves the administration of more than one test simultaneously. Individuals who receive a positive result for one or the other test are considered to have cancer. By identifying more true positives, as well as false positives, parallel testing has the effect of increasing the probability of detecting cancer (higher sensitivity) for a trade-off in specificity. In contrast, serial testing involves administering more than one test sequentially. Individuals who receive a positive test result on the first test are evaluated further with a second test; a second positive test result may then evoke a third test, and so forth, until a confirmatory diagnostic procedure is applied. Individuals who receive a positive test result on both the first and subsequent tests are considered to have cancer. By identifying more true negatives, as well as false negatives, serial testing has the effect of increasing the probability of identifying individuals without disease (higher specificity) at the expense of sensitivity. Alternatively, parallel versus serial testing can be evaluated by applying mathematical algorithms that use “or” versus “and” decision rules, and the tests can be given simultaneously. Two-sided McNemar’s tests were used to determine if significant differences in accuracy exist between testing algorithms using the statistical analyses system (SAS Institute). Univariate logistic regression was used to assess whether sEGFR concentrations (78) and CA125 levels are associated with EOC. Multivariate logistic regression models were used to assess confounding by age or menopausal status and to test for interactions between covariates (e.g., age, sEGFR, and CA125). Receiver Operating Characteristic curves (79), which plot sensitivity against 1 – specificity across all possible cutoff thresholds, were used to compare the ability of each logistic regression model to discern EOC cases from women with benign ovarian neoplasms, benign non-ovarian gynecologic conditions, and both benign groups combined. The receiver operating characteristic summary statistic called the area under the curve, which estimates the probability of correctly discerning a person with cancer from a person without cancer for all possible cutoff thresholds, was used to compare models.

**Results**

The Groups of Women Differ in Age and Menopausal Status. The 225 EOC patients in this study ranged in age from 24 to 87 years (median, 61 years): 35 of these women were classified as premenopausal, 183 as postmenopausal, and 7 as indeterminate. The 246 patients with benign ovarian neoplasms ranged in age from 18 to 88 years (median, 51 years): 108 of these women were classified as premenopausal, 123 as postmenopausal, and 15 as indeterminate. Lastly, the 253 patients with benign gynecologic conditions ranged in age from 18 to 84 years (median, 42 years); 187 of these women were classified as premenopausal, 53 as postmenopausal, and 13 as indeterminate. As such, the EOC cases are older than the patients with benign ovarian neoplasms (Wilcoxon rank-sum test, P < 0.0001), who, in turn, are older than the patients with benign non-ovarian gynecologic conditions (Wilcoxon rank-sum test, P < 0.0001). Accordingly, the patients with EOC are also older than the patients with benign non-ovarian gynecologic conditions (Wilcoxon rank-sum test, P < 0.0001). Thus, age and menopausal status may be potential confounders of the relationship between sEGFR concentrations or CA125 levels and EOC.

**Serum sEGFR Concentrations are Lower in Patients with EOC than in Women with Benign Gynecologic Conditions.** Patients with EOC have lower serum p110 sEGFR concentrations than women with either benign ovarian neoplasms or benign gynecologic conditions of non-ovarian origin (P < 0.0001 for both comparisons; Table 1; Fig. 1A). Moreover, serum sEGFR concentrations do not differ between women with benign ovarian neoplasms and benign non-ovarian gynecologic conditions (P = 0.1478). Following stratification based on menopausal status, we observe that sEGFR concentrations are lower in both premenopausal (P = 0.0007 for both comparisons) and postmenopausal (P < 0.0001 for both comparisons) EOC patients compared with women of identical menopausal status who either have benign ovarian neoplasms or benign gynecologic conditions of non-ovarian origin. And serum sEGFR concentrations do not differ between premenopausal (P = 0.4660) or postmenopausal (P = 0.3245) women with benign ovarian neoplasms and other benign gynecologic conditions. Furthermore, sEGFR concentrations do not differ between premenopausal and postmenopausal patients with either EOC (P = 0.9486), benign ovarian neoplasms (P = 0.3316), or other benign gynecologic conditions (P = 0.6645). Finally, comparison of serum sEGFR concentrations versus age shows no association between sEGFR concentrations and age for patients with EOC (P = 0.0133; Fig. 2A), benign ovarian neoplasms (P = 0.0140; Fig. 2B), or other benign gynecologic conditions (P = 0.0908; Fig. 2C).

Univariate logistic regression shows an association between log sEGFR concentration and EOC versus women with benign ovarian tumors, benign gynecologic conditions of non-ovarian origin (P < 0.0001 for all comparisons), and both benign groups combined, the odds of a 1 log unit decrease in serum p110 sEGFR concentration is 1.439 [95% confidence interval (CI), 1.27-1.51] times greater for women with EOC, compared with women with benign ovarian neoplasms (Wilcoxon rank-sum test, P = 0.9237; data not shown). The results for CA125 concentrations versus age for patients with EOC (P = 0.0274; menopausal status, P = 0.8232) show that menopausal status and age are not confounders or effect modifiers of the association between sEGFR and EOC versus benign gynecologic disease.
Interestingly, comparison of CA125 levels versus age reveals that CA125 levels: (a) increase with age in EOC patients \( (\rho = 0.2077; \ P = 0.0019; \ \text{Fig. 2D}) \), (b) tend to decrease with age for patients with benign ovarian neoplasms \( (\rho = -0.0959; \ P = 0.1336; \ \text{Fig. 2E}) \), and (c) decrease with age in women with benign non-ovarian gynecologic conditions \( (\rho = -0.1793; \ P = 0.0042; \ \text{Fig. 2G}) \). Consequently, we observe that CA125 levels decrease with age among patients with both benign ovarian and non-ovarian gynecologic conditions combined \( (\rho = -0.2048; \ P < 0.0001; \ \text{data not shown}) \). Taken together, these observations suggest that important menopausal status-disease (ovarian cancer versus benign disease) and age-disease interactions for serum CA125 levels may exist.

Univariate logistic regression models show an association between log CA125 level and EOC versus women with benign ovarian tumors, benign gynecologic conditions of non-ovarian origin, and both benign groups combined \( (P < 0.0001 \ \text{for all comparisons}) \); Table 2). When compared to women with benign ovarian neoplasm, women with benign non-ovarian gynecologic conditions, and both of these groups combined, the odds of a 1 log unit increase in CA125 level is 3.276 (95% CI, 2.58-4.16), 2.98 (95% CI, 1.95-2.71), and 2.864 (95% CI, 2.42, 3.39) times greater for women with EOC, respectively. Multivariate logistic regression models show that the association between log CA125 level and EOC is not confounded substantially by age on a continuous scale or menopausal status for either group of women with benign disease or both groups combined (confounding ≤10% for each comparison; Table 2). Moreover, there is no evidence for an interaction of menopausal status \( (Wald \ \chi^2 \text{ for log CA125 } \times \text{ menopausal status}) \) on the association between CA125 and EOC versus women with benign ovarian neoplasm \( (P = 0.9975) \) or women with benign non-ovarian gynecologic conditions \( (P = 0.2358) \), but there is evidence for effect modification by menopausal status on the association between CA125 and EOC versus both benign groups of women combined \( (P = 0.0965; \ \text{borderline significance; data not shown}) \). Although we do not observe an age-disease interaction for CA125 levels \( (Wald \ \chi^2 \text{ for log CA125 } \times \text{ age}) \) when using women with benign ovarian neoplasms as the comparison group \( (P = 0.1462) \), we do observe effect

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Median SEGFR (fmol/ml)</th>
<th>Range SEGFR (fmol/ml)</th>
<th>Wilcoxon Rank Sum ( P )-value</th>
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</thead>
<tbody>
<tr>
<td><strong>EOC</strong> ( (n = 225) )</td>
<td>1,822</td>
<td>ND-35,255</td>
<td>( P &lt; 0.0001 )</td>
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<tr>
<td><strong>Benign ovarian</strong> ( (n = 246) )</td>
<td>1,467</td>
<td>ND-20,895</td>
<td>( P = 0.1478 )</td>
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<td><strong>Benign gynecologic</strong> ( (n = 253) )</td>
<td>1,507</td>
<td>ND-24,165</td>
<td>( P = 0.1336 )</td>
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**Premenopause**

<table>
<thead>
<tr>
<th>Patient groups</th>
<th>Median CA125 (units/ml)</th>
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**Table 1. Comparison of serum sEGFR concentrations and CA125 levels among patients with EOC, benign ovarian neoplasms, and other benign gynecologic conditions of non-ovarian origin, before and after stratification based on menopausal status**
modification by age on the association between CA125 and EOC versus women with benign non-ovarian gynecologic conditions \( (P = 0.1094; P = 0.0825; \text{Fig. 3C}) \). Consequently, we also do not observe an association between sEGFR concentrations and CA125 levels among patients with benign ovarian and non-ovarian gynecologic conditions combined \( (P = 0.0458; P = 0.3072; \text{data not shown}) \). These findings suggest an important sEGFR-disease (ovarian cancer versus benign disease) interaction for serum CA125 levels. To test whether an interaction exists between sEGFR and CA125 on the association with EOC we performed multivariate logistic regression analysis with the terms log sEGFR, log CA125, and \((\log \text{sEGFR} \times \log \text{CA125})\) included in the model. We observe that serum sEGFR concentrations modify the association between CA125 levels \( (\text{Wald } \chi^2 \text{ for log sEGFR} \times \log \text{CA125}) \) and EOC when compared with benign ovarian neoplasms \( (P = 0.0813; \text{borderline significance}) \), benign non-ovarian gynecologic conditions \( (P = 0.0040) \), and both groups of benign disease combined \( (P = 0.0057; \text{data not shown}) \). These analyses show that sEGFR is an important effect modifier of the association between CA125 and EOC versus benign gynecologic disease. As such, variable cutoff thresholds for both biomarkers (i.e., sEGFR-dependent cutoff values for CA125 levels, and, reciprocally, CA125-dependent cutoff values for sEGFR concentrations) are appropriate when discerning EOC patients from women with benign ovarian neoplasms, benign non-ovarian gynecologic conditions, and both groups of women with benign disease combined.

Serum sEGFR Concentrations Modify the Association Between CA125 Levels and EOC. Comparison of p110 sEGFR concentrations versus CA125 levels reveals that sEGFR concentrations are inversely associated with CA125 levels in EOC patients \( (\rho = -0.3031; P < 0.0001; \text{Fig. 3A}) \), but not in women with benign ovarian neoplasms \( (P = 0.0167; P = 0.7939; \text{Fig. 3B}) \) or women with benign non-ovarian gynecologic conditions \( (P = 0.1094; P = 0.0825; \text{Fig. 3C}) \). Consequently, we also do not observe an association between sEGFR concentrations and CA125 levels among patients with benign ovarian and non-ovarian gynecologic conditions combined \( (P = 0.0458; P = 0.3072; \text{data not shown}) \). These findings suggest an important sEGFR-disease (ovarian cancer versus benign disease) interaction for serum CA125 levels. To test whether an interaction exists between sEGFR and CA125 on the association with EOC we performed multivariate logistic regression analysis with the terms log sEGFR, log CA125, and \((\log \text{sEGFR} \times \log \text{CA125})\) included in the model. We observe that serum sEGFR concentrations modify the association between CA125 levels \( (\text{Wald } \chi^2 \text{ for log sEGFR} \times \log \text{CA125}) \) and EOC when compared with benign ovarian neoplasms \( (P = 0.0813; \text{borderline significance}) \), benign non-ovarian gynecologic conditions \( (P = 0.0040) \), and both groups of benign disease combined \( (P = 0.0057; \text{data not shown}) \). These analyses show that sEGFR is an important effect modifier of the association between CA125 and EOC versus benign gynecologic disease. As such, variable cutoff thresholds for both biomarkers (i.e., sEGFR-dependent cutoff values for CA125 levels, and, reciprocally, CA125-dependent cutoff values for sEGFR concentrations) are appropriate when discerning EOC patients from women with benign ovarian neoplasms, benign non-ovarian gynecologic conditions, and both groups of women with benign disease combined.

Serum sEGFR Concentrations and CA125 Levels as Screening and Diagnostic Tests for EOC. To assess the individual and combined performance of serum p110 sEGFR concentrations and CA125 levels to detect patients with EOC, and to discern patients with EOC from patients with benign ovarian neoplasms and benign non-ovarian gynecologic conditions, we first examined graphs of sEGFR versus CA125 for each group of women to select optimal fixed cutoff thresholds for each biomarker and then calculated test sensitivity, specificity, and accuracy (Tables 3 and 4; Fig. 3). At fixed cutoff thresholds of \( \leq 1,000 \text{ fmol/mL sEGFR} \) and \( \geq 50 \text{ units/mL CA125} \), CA125 outperforms sEGFR, having greater accuracy to discern EOC patients from women with benign ovarian neoplasms \( (86.3\% \text{ versus } 63.9\%; \text{McNemar’s test, } P < 0.0001) \), benign non-ovarian gynecologic conditions \( (79.4\% \text{ versus } 61.5\%; \text{McNemar’s test, } P = 0.0059) \), and both benign groups of women combined \( (85.9\% \text{ versus } 62.7\%; \text{McNemar’s test, } P < 0.0001) \). Compared to the standard cutoff threshold of \( \geq 35 \text{ units/mL} \) recommended for CA125, a cutoff threshold of \( \geq 50 \text{ units/mL} \) CA125 is more accurate for discerning EOC patients from women with benign ovarian neoplasms \( (85.0\% \text{ versus } 86.3\%; \text{McNemar’s test, } P = 0.0002) \), benign non-ovarian gynecologic conditions \( (77.1\% \text{ versus } 79.4\%; \text{McNemar’s test, } P < 0.0001) \), and both benign groups combined \( (82.9\% \text{ versus } 85.9\%; \text{McNemar’s test, } P < 0.0001) \). Thus, a fixed cutoff threshold of \( \geq 50 \text{ units/mL} \) CA125 distinguishes EOC cases from women with benign gynecologic disease with greater accuracy than fixed cutoff thresholds of \( \leq 1,000 \text{ fmol/mL sEGFR} \) and \( \geq 35 \text{ units/mL CA125} \) as individual tests.

For serial testing, which requires that both sEGFR and CA125 are abnormal to classify a patient as having EOC, fixed cutoff thresholds of \( \leq 1,000 \text{ fmol/mL sEGFR} \) and \( \geq 50 \text{ units/mL CA125} \) discern patients with EOC from patients with benign ovarian neoplasms with 100% specificity (Fig. 3B), 50.9% sensitivity (Fig. 3A), and 76.7% accuracy (Tables 3 and 4). Using these cutoff thresholds, serial testing shows 97.6% specificity (Fig. 3C) and 75.8% accuracy for discerning EOC cases from patients with benign non-ovarian gynecologic conditions, and 98.8% specificity and 94.0% accuracy for all women with benign conditions combined; sensitivity remains at 50.9% for both comparisons. Interestingly, a fixed
cutoff threshold of ≥135 units/mL is necessary to achieve 100% specificity to discern EOC cases from women with benign ovarian neoplasms with CA125 alone; at this cutoff, sensitivity and accuracy are 58.1% and 80.1%, respectively (Tables 3 and 4). Using ≥135 units/mL as the cutoff threshold, CA125 shows 95.7% specificity and 78.1% accuracy for discerning EOC cases from patients with benign non-ovarian gynecologic conditions, and 97.8% specificity and 85.6% accuracy for all women with benign conditions combined; sensitivity remains at 58.1% for both comparisons. In contrast, parallel testing with fixed cutoff thresholds of 1,000 fmol/mL sEGFR and ≥50 units/mL CA125, which requires that sEGFR or CA125 are abnormal, increases sensitivity to 84.8% (Fig. 3D) at the expense of specificity and accuracy when EOC patients are compared to women with benign ovarian neoplasms (specificity, 63.4%; accuracy, 80.1%).

Table 2. Odds ratios (OR), 95% CI, and percent confounding (%) for a 1 log unit decrease in serum sEGFR concentration or a 1 log unit increase in CA125 level among EOC cases versus women with benign ovarian neoplasms, women with benign gynecologic conditions of non-ovarian origin, and both groups of women with benign disease combined

<table>
<thead>
<tr>
<th>sEGFR (1 log unit decrease)</th>
<th>EOC versus Benign Ovarian Conditions, OR (CI) %</th>
<th>Wald $\chi^2$</th>
<th>EOC versus Benign Gynecologic Conditions, OR (CI) %</th>
<th>Wald $\chi^2$</th>
<th>EOC versus all Benign Conditions, OR (CI) %</th>
<th>Wald $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>1.439 (1.29-1.61)</td>
<td>&lt;0.0001</td>
<td>1.362 (1.23-1.51)</td>
<td>&lt;0.0001</td>
<td>1.382 (1.27-1.51)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>1.451 (1.29-1.62)</td>
<td>&lt;0.0001</td>
<td>1.366 (1.20-1.55)</td>
<td>&lt;0.0001</td>
<td>1.403 (1.28-1.54)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Menopause-adjusted</td>
<td>1.460 (1.30-1.54)</td>
<td>&lt;0.0001</td>
<td>1.361 (1.20-1.54)</td>
<td>&lt;0.0001</td>
<td>1.403 (1.28-1.54)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CA125 (1 log unit increase)</th>
<th>EOC versus Benign Ovarian Conditions, OR (CI) %</th>
<th>Wald $\chi^2$</th>
<th>EOC versus Benign Gynecologic Conditions, OR (CI) %</th>
<th>Wald $\chi^2$</th>
<th>EOC versus all Benign Conditions, OR (CI) %</th>
<th>Wald $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>3.276 (2.58-4.16)</td>
<td>&lt;0.0001</td>
<td>2.298 (1.95-2.71)</td>
<td>&lt;0.0001</td>
<td>2.864 (2.42-3.39)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age-adjusted</td>
<td>3.153 (2.48-4.01)</td>
<td>&lt;0.0001</td>
<td>2.122 (1.76-2.56)</td>
<td>&lt;0.0001</td>
<td>2.787 (2.33-3.34)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Menopause-adjusted</td>
<td>3.282 (2.49-4.07)</td>
<td>&lt;0.0001</td>
<td>2.238 (1.83-2.73)</td>
<td>&lt;0.0001</td>
<td>2.853 (2.36-3.44)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Wald $\chi^2$ P value for log sEGFR or log CA15 in each logistic regression model.
73.6%; Fig. 3E), benign non-ovarian gynecologic conditions (specificity, 47.8%; accuracy, 65.2%; Fig. 3F), and benign non-ovarian gynecologic conditions (specificity, 55.5%; accuracy, 64.6%). Therefore, a fixed cutoff threshold of $z_{135}$ units/mL CA125 discerns EOC cases from women with benign ovarian neoplasms (80.1% versus 76.7%), benign non-ovarian gynecologic conditions (78.1% versus 75.8%), and both benign groups combined (85.6% versus 84.0%) with greater accuracy than serial testing with both sEGFR and CA125 using fixed cutoff thresholds of $V_{1,000}$ fmol/mL sEGFR and $z_{50}$ units/mL, respectively (McNemar’s test, $P < 0.0001$ for all comparisons).

However, parallel testing with $V_{1,000}$ fmol/mL sEGFR and $z_{50}$ units/mL CA125 maximizes the ability to detect EOC (84.8% sensitivity). Multivariate logistic regression analyses indicate that variable cutoff thresholds are appropriate when using sEGFR and CA125 together. Inspection of sEGFR versus CA125 plots for women with benign ovarian neoplasms shows that a fixed cutoff threshold of $z_{30}$ units/mL CA125 may be useful for sEGFR concentrations from nondetectable values to 99 fmol/mL (Fig. 3G-I), where $y = s$EGFR concentration and $x = CA125$ level (diagonal line). Spearman’s rank-order correlation coefficient ($\rho$) and corresponding $P$ value are given for each comparison of sEGFR concentration versus CA125 level (A, B, and C).

Figure 3. Serum sEGFR concentrations versus CA125 levels in patients with EOC (A, D, and G), benign ovarian neoplasms (B, E, and H), and benign non-ovarian gynecologic conditions (C, F, and I) are compared. Test sensitivity (A, D, and G) and specificity (B, C, E, F, H, and I) are shown (highlighted areas) for “in series” (A, B, and C) and “in parallel” (D, E, and F) testing with fixed cutoff thresholds of $\leq 1,000$ fmol/mL sEGFR (horizontal line) and $\geq 50$ units/mL CA125 (vertical line), and for “in series” testing with variable cutoff thresholds (G, H, and I). Variable cutoff thresholds were selected as follows: fixed at $\geq 30$ units/mL CA125 between nondetectable values to 99 fmol/mL sEGFR (horizontal line) and variable for sEGFR concentrations $\geq 100$ fmol/mL according to the formula: $y = 0.0037x^3$, where $y = s$EGFR concentration and $x = CA125$ level (diagonal line). Spearman’s rank-order correlation coefficient ($\rho$) and corresponding $P$ value are given for each comparison of sEGFR concentration versus CA125 level (A, B, and C).
and tumors, benign non-ovarian gynecologic conditions, and both discerns patients with EOC from women with benign ovarian

\[ P = 0.0028 \] and benign gynecologic conditions of non-ovarian origin (neoplasms (Fig. 4D, E, and F; arrow). In contrast, a fully reduced model incorporating only log CA125 correctly distinguishes patients with EOC from women with benign ovarian tumors (Fig. 4A), benign non-ovarian gynecologic conditions (Fig. 4B), and both benign groups of women combined (Fig. 4C) with 85.6% (95% CI, 81.9-89.3%), 80.8% (95% CI, 76.6-85.0%), and 83.2% (95% CI, 79.4-86.9%) probability across all cutoff thresholds, respectively. Sensitivity for detecting EOC when compared with each of these respective groups of women is ~70%, 65%, and 60%, where the full model converges to 100% specificity (Fig. 4D, E, and F; arrow). Multivariate logistic regression, which can model serial testing with age- and sEGFR-dependent CA125 cutoff thresholds simultaneously, therefore, optimizes test accuracy to discern patients with EOC from women with benign ovarian and non-ovarian gynecologic conditions.

To better understand the individual and combined utility of sEGFR and CA125 as screening tests for early stage EOC, we examined the sensitivity of each screening algorithm to detect stage I/II versus stage III/IV EOC (Table 4). At a cutoff threshold of ≤1,000 fmol/mL, sEGFR detected 45.5% of stage I/II and 67.2% of stage III/IV cancers, whereas CA125 only detected 21.4%, 16.7%, and 9.5% of stage I/II cancers compared with 87.2%, 86.0%, and 69.3% of stage III/IV cancers at cutoff thresholds of ≥35, ≥50, and ≥135 units/mL, respectively. Using fixed cutoff thresholds of ≤1,000 fmol/mL sEGFR and ≥50 units/mL CA125, serial testing with both biomarkers detected 4.8% of stage I/II and 61.5% of stage III/IV cancers, whereas parallel testing detected 56.8% of stage I/II and 91.7% of stage III/IV cancers. Compared to serial testing with fixed cutoff thresholds, serial testing with variable sEGFR and CA125 cutoff thresholds improved the detection of stage I/II and stage III/IV cancers from 4.8% to 14.3% and 61.5% to 80.4%, respectively. Finally, the full logistic regression model, which applies both age-dependent and sEGFR-dependent CA125 cutoff thresholds, shows 11.8% and 72.0% sensitivity in detecting stage I/II and stage III/IV EOC, respectively, where the model converges to 100% specificity for the comparison of EOC cases to women with benign ovarian and non-ovarian gynecologic conditions.

EOC cases compared with patients with benign non-ovarian gynecologic conditions

<table>
<thead>
<tr>
<th>Cutoff thresholds</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1,000 fmol/mL sEGFR</td>
<td>153/253 (60.5%)</td>
<td>294/478 (61.5%)</td>
</tr>
<tr>
<td>≤35 units/mL CA125</td>
<td>200/253 (79.1%)</td>
<td>366/475 (77.1%)</td>
</tr>
<tr>
<td>≤50 units/mL CA125</td>
<td>215/253 (85.0%)</td>
<td>377/475 (79.4%)</td>
</tr>
<tr>
<td>≤135 units/mL CA125</td>
<td>242/253 (95.7%)</td>
<td>371/475 (78.1%)</td>
</tr>
<tr>
<td>≤1,000 fmol/mL sEGFR or ≤50 units/mL CA125</td>
<td>121/253 (47.8%)</td>
<td>311/475 (65.2%)</td>
</tr>
<tr>
<td>≤50 units/mL CA125</td>
<td>121/253 (47.8%)</td>
<td>311/475 (65.2%)</td>
</tr>
<tr>
<td>γ Variable sEGFR concentrations and CA125 levels</td>
<td>228/253 (90.1%)</td>
<td>379/475 (79.8%)</td>
</tr>
</tbody>
</table>

EOC cases compared with all patients with benign disease

<table>
<thead>
<tr>
<th>Cutoff thresholds</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1,000 fmol/mL sEGFR</td>
<td>313/499 (62.7%)</td>
<td>454/722 (62.7%)</td>
</tr>
<tr>
<td>≤35 units/mL CA125</td>
<td>432/499 (86.6%)</td>
<td>598/721 (82.9%)</td>
</tr>
<tr>
<td>≤50 units/mL CA125</td>
<td>457/499 (91.6%)</td>
<td>619/721 (85.9%)</td>
</tr>
<tr>
<td>≤135 units/mL CA125</td>
<td>488/499 (97.8%)</td>
<td>617/721 (85.6%)</td>
</tr>
<tr>
<td>≤1,000 fmol/mL sEGFR or ≤50 units/mL CA125</td>
<td>277/499 (55.5%)</td>
<td>467/723 (64.6%)</td>
</tr>
<tr>
<td>≤50 units/mL CA125</td>
<td>277/499 (55.5%)</td>
<td>467/723 (64.6%)</td>
</tr>
<tr>
<td>γ Variable sEGFR concentrations and CA125 levels</td>
<td>474/499 (95.0%)</td>
<td>625/721 (86.7%)</td>
</tr>
</tbody>
</table>

NOTE: TP, true positive; FP, false positive; TN, true negative; FN, false negative.

Sensitivity = TP / (TP + FN); specificity = TN / (TN + FP); accuracy = (TP + TN) / (TP + TN + FP + FN); \( \chi^2 \) test, P < 0.0001 for all comparisons. Serial testing with variable sEGFR and CA125 cutoff thresholds, therefore, increases test accuracy to discern patients with EOC from women with benign ovarian and non-ovarian gynecologic conditions.

Multivariate logistic regression indicates that age-dependent cutoff thresholds are appropriate for CA125. To evaluate both the age-dependent and sEGFR-dependent cutoff thresholds for serum CA125 levels simultaneously, we fit a logistic regression model that included the following terms: log sEGFR, log CA125, age, (log sEGFR \* log CA125), and (log CA125 \* age; Table 5). Each of the interaction terms (log sEGFR \* log CA125 and log CA125 \* age) is significant when the model is used to compare patients with EOC to women with benign ovarian neoplasms (P = 0.0395 and P = 0.0640; borderline significance), benign gynecologic conditions of non-ovarian origin (P = 0.0048 and P = 0.0035), and both benign groups of women combined (P = 0.0028 and P = 0.0004). Moreover, the full model better discriminates patients with EOC to women with benign ovarian tumors, benign non-ovarian gynecologic conditions, and both benign groups combined than a reduced model that includes only log CA125 (~2 log likelihood ratio \( \chi^2 \) test, P < 0.0005 for all comparisons; data not shown). Receiver operating characteristics curves show that the full logistic regression model has 87.2% (95% CI, 83.6-90.7%), 90.1% (95% CI, 87.0-93.2%), and 87.4% (95% CI, 84.1-90.8%) probability of correctly discerning EOC cases from patients with benign ovarian neoplasms (Fig. 4D), benign non-ovarian gynecologic conditions (Fig. 4E), and both groups of women combined (Fig. 4F) across all cutoff thresholds, respectively. Sensitivity for detecting EOC when compared with each of these respective groups of women is ~70%, 65%, and 60%, where the full model converges to 100% specificity (Fig. 4D, E, and F; arrow).
Table 5. Multivariate logistic regression modeling of women with EOC compared to women with benign ovarian tumors, benign gynecologic conditions of non-ovarian origin, and both benign groups combined

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Benign ovarian</th>
<th></th>
<th>Benign gynecologic</th>
<th>Both benign groups</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
<td>Wald $\chi^2$</td>
<td>$P$</td>
<td>Maximum</td>
<td>Wald $\chi^2$</td>
</tr>
<tr>
<td></td>
<td>likelihood</td>
<td></td>
<td></td>
<td>likelihood</td>
<td></td>
</tr>
<tr>
<td>Log sEGFR</td>
<td>0.2795</td>
<td>1.2453</td>
<td>0.2644</td>
<td>0.4089</td>
<td>3.2308</td>
</tr>
<tr>
<td>Log CA125</td>
<td>1.2168</td>
<td>2.6057</td>
<td>0.1065</td>
<td>0.4789</td>
<td>0.5893</td>
</tr>
<tr>
<td>Age</td>
<td>-0.0274</td>
<td>0.8917</td>
<td>0.399</td>
<td>-0.1949</td>
<td>7.9452</td>
</tr>
<tr>
<td>Log sEGFR $\times$ log CA125</td>
<td>-0.1671</td>
<td>4.2376</td>
<td>0.0395</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log CA125 $\times$ age</td>
<td>0.0184</td>
<td>3.4313</td>
<td>0.0640</td>
<td>0.0299</td>
<td>8.5229</td>
</tr>
</tbody>
</table>

Discussion

Age is a well-established risk factor for EOC (80). Age is also a surrogate parameter for menopause-related changes in circulating steroid and gonadotropin hormone concentrations in women, which decrease or increase after menopause, respectively (81). It is noteworthy that serum p110 sEGFR concentrations are negatively associated with both follicle-stimulating hormone and luteinizing hormone concentrations in healthy women (76). Moreover, serum sEGFR concentrations exhibit an age-disease interaction, decreasing with age in healthy women, but not in patients with EOC (74). Altogether, these observations suggest that gonadotropin hormones may regulate serologic sEGFR, and that this regulatory pathway may be altered in patients with EOC. In this study, we report that both age-disease and sEGFR-disease interactions exist for serum CA125 levels, when comparing EOC to benign gynecologic disease. Specifically, we observe that serum CA125 levels increase with age in EOC patients, but decrease with age in patients with benign gynecologic conditions of ovarian and non-ovarian origin. We also observe that serum CA125 levels increase inversely with sEGFR concentrations in EOC patients, but show no association in patients with benign ovarian and non-ovarian gynecologic conditions. Several reports have shown that CA125 levels may be elevated in EOC cases more than 2 years before they present with clinically overt disease (40, 82), and that elevated serum CA125 levels may be an endogenous risk factor (or perhaps a surrogate marker of exposure to a risk factor) for EOC (83, 84). Taken together, these observations suggest that a causal relationship may exist between age and the expression of gonadotropic peptide hormones, p110 sEGFR and CA125 in the development of EOC. Laboratory cell culture experiments, animal model studies, and additional epidemiologic studies will be needed to test the hypothesis that dysregulated expression of this nexus of molecules is associated with the development and pathogenesis of ovarian cancer.

The major obstacle confronting the development of a population-based screening program for EOC remains the low prevalence of this disease in the general population, estimated to range between 38 to 47 cases per 100,000 for women of average risk in the general population and 254 cases per 100,000 for women of higher risk with a family history of EOC (85). Assuming a disease prevalence of 40 cases per 100,000 (0.04%) for women of average risk, a screening program would require a sensitivity of 100% and specificity of 99.64% to achieve a positive predictive value of 10% (Table 6). Yet, if the screened population were limited to women with a family history of ovarian cancer where disease prevalence is about 250 cases per 100,000 (0.25%), a screening program having 100% sensitivity and 97.74% specificity would achieve the minimally acceptable goal of 10% positive predictive value; that is, one ovarian cancer would be detected for every 10 surgical procedures (33, 86). These calculations show that to make screening for EOC practical, we must devise a highly sensitive and specific screening program, and we must apply this program to a subgroup of women who are characterized by high disease prevalence. Moreover, this subgroup must correspond to women who are at risk of developing sporadic, nonfamilial EOC, which represent 90% of all EOC cases. This is an enormous challenge that concerns the “in parallel” versus “in series” rules of testing. How can we design a screening program to achieve our goals given these mathematical laws, and simultaneously solve the problem of low disease prevalence? The solution to the puzzle must involve identifying a subgroup of women from the general population who exhibit an ovarian cancer phenotype at interval testing, and who are characterized by a disease prevalence that is higher than that of women with a family history of ovarian cancer. If we can identify a subgroup of women where disease prevalence is 500 cases per 100,000 (0.5%) or preferably 1,000 cases per 100,000 (1.0%) from among the general population, we could attain 10% positive predictive value with a screening program characterized by 60% sensitivity and 97.29% specificity or even 60% sensitivity and 94.55% specificity, respectively (Table 6). Interestingly, if a subgroup with a prevalence of 0.5% (i.e., 40 cases per 8,000) or even better 1.0% (i.e., 40 cases per 4,000) could be identified from the general population, only 8,000 or 4,000 of every 100,000 women would need to undergo secondary, more costly, and thorough screening procedures, respectively. To identify a subgroup of women characterized by an ovarian cancer phenotype and high disease prevalence, we propose that screening programs for EOC evaluate multiple biomarkers using “in parallel” testing to drive sensitivity toward 100% at the expense of specificity as a crucial first step. This key step of “proteomic triage” would be expected to reduce the size of the target subpopulation for follow-up by “diagnostic triage” with other procedures such as transvaginal sonography with morphologic indexing (87) and additional biomarkers using “in series” testing algorithms to impel specificity toward 100%, although sacrificing some sensitivity.

We illustrate the concepts of proteomic triage and diagnostic triage with multiple biomarkers using the data of p110 sEGFR concentrations and CA125 levels presented in this study. At a cutoff threshold of ≤1,000 fmol/mL, sEGFR concentrations would have detected 45.5% of the stage I/II ovarian cancers (Table 4). Although lower than expected when compared with other published reports (38–41), CA125 levels would have detected just 21.4% of stage I/II ovarian cancers at a cutoff threshold of ≥35 units/mL. In contrast, “in parallel” testing using fixed thresholds of ≤1,000 fmol/mL sEGFR and ≥30 units/mL CA125 would have detected 56.8% of stage I/II ovarian cancers by proteomic triage. Using the “in parallel” screening algorithm, 36.6% and 52.2% of patients with benign ovarian neoplasms and benign gynecologic non-ovarian gynecologic conditions combined (data not shown). Parallel testing with fixed cutoff thresholds of ≤1,000 fmol/mL sEGFR and ≥50 units/mL CA125, therefore, maximizes the ability to detect stage I/II EOC (56.8% sensitivity).
Figure 4. Receiver-operating characteristic curves comparing women with EOC versus women with benign ovarian neoplasms (A), benign non-ovarian gynecologic conditions (B), and both benign groups of women combined (C) are shown for the completely reduced model, which includes only log CA125 as a continuous variable. Receiver operating characteristic curves comparing women with EOC versus women with benign ovarian neoplasms (D), benign non-ovarian gynecologic conditions (E), and both benign groups of women combined (F) are shown for the full logistic regression model, which includes log sEGFR, log CA125, age, (log sEGFR \times log CA125), and (log CA125 \times age) as continuous variables. The area under the curve and 95% CI is given for each receiver operating characteristic curve. Arrows, sensitivity where each model converges to 100% specificity.
Table 6. Test sensitivity and specificity necessary to achieve a positive predictive value of 10% given a disease prevalence of 40 cases per 100,000 (0.04%), 250 cases per 100,000 (0.25%), 500 cases per 100,000 (0.50%), or 1,000 cases per 100,000 (1.0%).

<table>
<thead>
<tr>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence</td>
<td></td>
</tr>
<tr>
<td>0.04%</td>
<td>99.78</td>
</tr>
<tr>
<td>0.25%</td>
<td>99.77</td>
</tr>
<tr>
<td>0.50%</td>
<td>99.75</td>
</tr>
<tr>
<td>1.00%</td>
<td>99.73</td>
</tr>
</tbody>
</table>

CA125, e.g., MUC1 mucin (88), CA50 (89), osteopontin (90), procollagen type III amino-terminal propeptide (91), soluble interleukin-2 receptor (92), urinary human chorionic gonadotropin core fragment (93), and urinary soluble urokinase-type plasminogen activator receptor (94). Future research, therefore, should focus on identifying the most useful combination of biomarkers from the repertoire currently available and still under discovery. Given these caveats and limitations, a well-designed prospective case-control study will be needed to confirm that the combination of serum sEGFR and CA125 may be useful as screening and diagnostic biomarkers of EOC. Such a study should include incident cases with EOC, benign ovarian neoplasms, and other benign gynecologic conditions, as well as healthy asymptomatic women from the same source population. We conclude that the combination of serum sEGFR and CA125 warrant further investigation with additional biomarkers, and transvaginal sonography as potential screening and diagnostic modalities of ovarian cancer.

Acknowledgments

We wish to thank Drs. Marites Buenafe, James Gurney, Martin McIntosh, Jill Reiter, and John van Nagell for critically reviewing this manuscript. Some serum samples used in this study were provided by Dr. Stephen Qualman, Director, Cooperative Human Tissue Network, Gynecologic Oncology Group Tissue Bank, which is supported by National Cancer Institute grants of the Gynecologic Cancer Group Tissue Bank and Administrative Office (CA 27298), and the Gynecologic Oncology Group Statistical and Data Center (CA 37517); other investigators may have received samples from the same subjects.

References


**Correction**

In an article in the February 2005 issue of *Cancer Epidemiology, Biomarkers & Prevention* (1), the title of the article contained the following error: “sEGFR” was abbreviated as “SEG-FR.” The correct title follows.

**Soluble Epidermal Growth Factor Receptor (sEGFR) and Cancer Antigen 125 (CA 125) as Screening and Diagnostic Tests for Epithelial Ovarian Cancer**

**Reference**

Soluble Epidermal Growth Factor Receptor (SEG-FR) and Cancer Antigen 125 (CA125) as Screening and Diagnostic Tests for Epithelial Ovarian Cancer

Andre T. Baron, Cecelia H. Boardman, Jacqueline M. Lafky, et al.


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