Soluble Epidermal Growth Factor Receptor (sEGFR/sErbB1) as a Potential Risk, Screening, and Diagnostic Serum Biomarker of Epithelial Ovarian Cancer[1]

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Abstract

Epithelial ovarian cancer (EOC) is the leading cause of death from gynecologic malignancies in the United States, for which risk assessment, screening, and diagnostic tests are needed. We have shown previously that women with stage III/IV EOC have lower serum p110 sEGFR/sErbB1 (Soluble Epidermal Growth Factor Receptor) concentrations than healthy women. Here, we show that serum p110 sEGFR/sErbB1 is the product of a 3-kb *EGFR/ERBB1* alternate transcript. We report that serum sEGFR concentrations in stage I/II and stage III/IV EOC patients are significantly lower than in healthy women, and that serum sEGFR concentrations are not associated with disease stage or tumor grade. Logistic regression models show that: (*a*) lower serum sEGFR concentrations are associated significantly with a greater risk of EOC; (*b*) the risk associated with lower serum sEGFR concentrations is reduced by older age or menopause; and (*c*) age- or menopausal status-specific cutoff values for sEGFR concentration are appropriate. Receiver operating characteristic curves indicate that: (*a*) serum sEGFR concentrations are more effective in discerning stage III/IV than stage I/II EOC cases from healthy women; and (*b*) sEGFR concentrations have an 89% probability of correctly discerning EOC patients from healthy women when accounting for effect modification by age. By maintaining a test specificity of ~95% across strata of age or menopausal status with appropriate cutoff values, we observe that sEGFR concentrations are most useful for detecting stage I/II (sensitivity: 64–67%) and stage III/IV (sensitivity: 75–81%) EOC in young, premenopausal women. We conclude that serum sEGFR concentrations warrant additional investigation in the risk assessment, early detection, and/or diagnosis of EOC.

Introduction

EOC[4] is a serious health problem for women. In the United States, EOC is the fifth most common cancer in women and is the leading cause of death from gynecologic malignancies (1). Approximately 23,000 new cases and 13,900 deaths of EOC are estimated to occur in the United States in 2002. The high mortality rate of EOC occurs because most women are diagnosed with advanced disease (stage III/IV), which has a 5-year survival rate of just 15–20% (2, 3). Late-stage diagnosis is a consequence of vague nonspecific symptoms, such as abdominal discomfort and bloating, dyspepsia, and urinary frequency, which do not alert women or their physicians to the presence of this silent cancer (4, 5). With the exception of family history or germ-line *BRCA1/2* mutations, clearly defined risk factors that might be used to recommend increased surveillance or prophylactic surgery for high-risk individuals do not exist for EOC; this fact negatively impacts on the mortality rate of EOC cases that develop sporadically (6, 7). Although clinical tools such as medical history, pelvic examination, ultrasonography, and serum CA-125 values aid in distinguishing EOC from benign adnexal masses, none of these tools are truly diagnostic (4). And, whereas the survival rate for stage I EOC patients is ~95% (8), no definitive screening test for early-stage EOC has been developed yet (9). Given these limitations, EOC patients may not be diagnosed until they are already in the operating room or after a definitive pathological evaluation has been completed postoperatively. In this situation, the patient with EOC may not receive a thorough initial cytoreductive staging operation, and subsequently may be referred to a gynecologic oncologist for a second staging operation (10). Consequently, only 35% and 52% of patients undergoing a primary surgery for

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[4] The abbreviations used are: EOC, epithelial ovarian cancer; EGFR, epidermal growth factor receptor; sErbB, soluble ErbB; ALISA, acridinium-linked immunosorbent assay; MAb, monoclonal antibody; NCI, National Cancer Institute; GOG, Gynecologic Oncology Group; ALBB, acridinium-linked immunosorbent assay blocking buffer; RLU, relative light unit; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; OR, odds ratio; BDL, biological detection limit; SD, standard deviation.

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an adnexal mass that may be EOC receive an adequate procedure from a general surgeon or gynecologic surgeon, respectively (11). Because the information obtained from a comprehensive staging operation is used to select additional treatment, these circumstances may adversely impact the morbidity and mortality associated with EOC, additionally emphasizing the critical need for a risk assessment, screening, and preoperative diagnostic test for this disease.

Given these clinical challenges, much recent effort has been placed on the development of new screening and diagnostic methods for EOC through the NCI Early Detection Research Program (12, 13). In this regard, soluble isoforms of the *ERBB* proto-oncogene family, which encodes four homologous receptor tyrosine kinases known as EGFR/ErbB1 (HER1), ErbB2 (HER2/*neu*), ErbB3 (HER3), and ErbB4 (HER4), are being explored as serum biomarkers with potential utility in the risk assessment, screening, and diagnosis of cancer (14–17). Soluble ErbB (sErbB) isoforms that embody extensive portions of the receptor extracellular domain exist for all four members of this receptor family. sErbB isoforms are produced either by proteolytic cleavage of the full-length receptor (18, 19) or by alternate splicing of mRNA transcripts (20–31). Our laboratory previously has cloned and characterized a 3-kb alternate mRNA transcript of the human *EGFR/ERBB1* proto-oncogene from human term placenta (31). This alternate mRNA predicts a protein product that is homologous with the extracellular domain of EGFR to amino acid residue 603; this sequence is followed by 78 unique COOH-terminal amino acids. Heterologous expression studies demonstrate that the 3-kb transcript is translated into a 110-kDa sEGFR/sErbB1 *N*-linked glycoprotein. We also have developed a sensitive ALISA that quantifies a 110-kDa sEGFR isoform in human sera (32). Moreover, we have demonstrated that serum sEGFR concentrations in women with advanced stage III/IV EOC are significantly lower than sEGFR concentrations in healthy women (33). These data suggested that low serum sEGFR concentrations might be useful biomarkers of EOC. We reported recently that serum sEGFR concentrations are inversely associated with age in healthy women and, consequently, that sEGFR concentrations are higher in premenopausal women compared with postmenopausal women (34). Accordingly, age and menopausal status may be potential confounders or effect modifiers of the relationship between serum sEGFR concentrations and EOC.

To understand better the relationship between circulating p110 sEGFR and the alternate 3-kb *EGFR/ERBB1* transcript, we purified this protein from human serum and subjected it to enzymatic digestion with trypsin followed by mass spectroscopic analyses. Our studies demonstrate that serum p110 sEGFR is the product of the 3-kb *EGFR/ERBB1* alternate transcript. To explore the potential clinical utility of this sEGFR isoform in the risk assessment, screening, and/or diagnosis of EOC, we compared preoperative serum sEGFR concentrations in 225 women with stage I-IV EOC to 144 healthy women. Data reported herein support a role for serum sEGFR concentrations in the risk assessment, early detection, and/or diagnosis of EOC.

Materials and Methods

Preparation of Anti-EGFR Immunoadsorbent Affinity Column. MAb 528 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) agarose resin was prepared using an AminoLink Plus immobilization kit (Pierce Chemical, Rockford, IL) according to the manufacturer's instructions. Anti-EGFR MAb 528 at 1.0 mg/ml was incubated with 2 ml of activated agarose resin (pH 7.2) for 6 h at room temperature. MAb 528-coupled resin was separated from unbound antibody by gravity. To assess coupling efficiency the concentration of unbound MAb 528 in binding solution was determined by Coomassie Plus Protein Assay (Pierce Chemical) before and after completion of the coupling reaction. A coupling efficiency >97% was achieved.

Purification of p110 sEGFR and Mass Spectrometry of Tryptic Peptides. Serum p110 sEGFR protein was purified as described previously by Gill and Weber (35) with some modifications. Briefly, 1 liter of human serum was equilibrated with Buffer A [20 mM HEPES (pH 7.4), 1 mM EDTA, 6 mM 2-mercaptoethanol, 0.05% Triton X-100, 10% glycerol, and 130 mM NaCl] supplemented with protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, 5 μg/ml aprotinin, and 1 mM EDTA) and cycled through a 2-ml anti-EGFR MAb 528-agarose column overnight at 4°C. The column was washed sequentially with Buffer A, Buffer A containing 1 M NaCl, and Buffer A containing 1 M urea at 4°C. Bound p110 sEGFR protein was eluted from the MAb 528-agarose column with Buffer A containing 6 M urea. Column fractions containing p110 sEGFR were pooled, dialyzed against 50 mM Trizma buffer (pH 7.4), containing 1 mM 2-mercaptoethanol and 0.1% Triton X-100, and separated additionally with a MonoQ anion exchange column using a Fast Performance Liquid Chromatography system (Pharmacia Biotech, Inc., Piscataway, NJ). Bound proteins were eluted from the MonoQ column with a 0–1 M linear gradient of NaCl in 50 mM Trizma buffer (pH 7.4), containing 1 mM 2-mercaptoethanol and 0.1% Triton X-100. MonoQ column fractions containing p110 sEGFR were pooled, concentrated, and exchanged into Buffer A by ultrafiltration using a Centricon YM-30 filter unit (Millipore Corporation, Bedford, MA), and resolved by SDS-PAGE. Serum sEGFR was excised from a preparative SDS-polyacrylamide electrophoretic gel, digested with trypsin, and subjected to microsequence analysis using Matrix Assisted Laser Desorption/Ionization-Time of Flight mass spectrometry by Borealis Biosciences, Inc. (Toronto, Ontario, Canada).

SDS-PAGE and Western Immunoblot Analyses. To assess the separation of p110 sEGFR from constituent serum proteins, chromatographic column fractions were analyzed by SDS-PAGE and Western immunoblot using methods described previously by Baron *et al.* (32). Western immunoblots were probed with anti-EGFR MAb 15E11 (36), which specifically recognizes the extracellular domain of EGFR, followed by incubation with a peroxidase-conjugated rabbit antimouse IgG1-specific secondary antibody. Detection of sEGFR was achieved by enhanced chemiluminescence with luminol (Amersham Life Science, Arlington Heights, IL).

Serum Samples. Serum samples were collected from 144 healthy women, between 1981 and 1984, by Mayo Medical Laboratories, Department of Laboratory Medicine and Pathology, in accordance with a Mayo Foundation Institutional Review Board-approved Normal Values Study as described previously (32). All of the serum samples were stored at −70°C. Menopausal status for this group of healthy women was determined as reported previously (34).

Preoperative serum samples from women with EOC were identified and obtained from repositories at the Mayo Clinic, Department of Gynecologic Surgery; the NCI, Cooperative Human Tissue Network, GOG Ovarian Tumor Bank; and the NCI Ovarian Cancer Early Detection Program at Northwestern University after obtaining Institutional Review Board approval. Serum samples were considered preoperative if they were col-

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lected within 30 days before primary cytoreductive surgery. Patients with a prior diagnosis of EOC who had received previous cytoreductive surgery, radiation, or chemotherapy were excluded from this study. Serum samples from women scheduled for gynecologic surgery at the Mayo Clinic between 1985 and 1994 were collected and stored to study the reproducibility of CA-125 measurements in women with EOC (37–40). Eighteen preoperative serum samples from women with EOC were identified from the Mayo repository as described previously (33). Preoperative serum samples (137) were obtained from women with EOC in accordance with GOG protocol #136 entitled, "Acquisition of human gynecologic specimens and serum to be used in studying the causes, diagnosis, prevention and treatment of cancer." These GOG serum specimens were collected between 1992 and 1998. Lastly, 70 preoperative serum samples were obtained from women with EOC through the NCI Ovarian Cancer Early Detection Program between 1999 and 2001. Information regarding patient age, menopausal status, International Federation of Gynecology and Obstetrics disease stage, tumor histological subtype, and tumor grade was made available from the Mayo Clinic, GOG, and NCI Ovarian Cancer Early Detection Program databases.

sEGFR/sErbB1 ALISA. Serum p110 sEGFR concentrations were determined by ALISA as outlined by Baron *et al.* (32, 33). Initially, all of the sera were diluted 1:10 in ALBB and assayed in duplicate in three separate trials. Serum samples yielding RLUs below the linear range of the standard curve of the assay were reassayed either undiluted or diluted 1:5 in ALBB, whereas serum samples yielding RLUs above the linear range of the standard curve of the assay were reassayed either diluted 1:20 or 1:50 in ALBB. For each trial, the mean RLU 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 SDs above the zero calibrator) for the ALISAs performed in this study was 7.5 fmol/ml sEGFR. The ALISA was partially automated using a BIOMEK 1000 laboratory workstation (Beckman-Coulter, Fullerton, CA). Acridinium decomposition was measured with a microplate luminometer (model LB 96P; EG&G Berthold Analytical Instruments, Nashua, NH).

Statistical Analysis. Descriptive statistics were calculated, and the Wilcoxon rank-sum test was used to determine whether significant differences in sEGFR concentrations exist between healthy women and women with EOC, before and after stratification based on menopausal status, and among EOC cases after stratification based on stage or tumor grade. Spearman's rank-order correlation coefficient (rho) was used to determine whether associations exist between sEGFR concentrations and age in healthy women or EOC cases, and among disease stage, tumor grade, age, and menopausal status in EOC cases. Descriptive statistics also were calculated for menopausal status- and age-matched EOC cases and controls, and the Wilcoxon signed-rank test was used to determine whether significant differences in sEGFR concentrations exist between matched groups. Cases with stage I/II or stage III/IV disease were matched randomly, one-to-one, on the basis of menopausal status and age (±2 years) to healthy women.

Univariate and multivariate logistic regression models were used to assess whether p110 sEGFR concentrations are associated with disease stage, tumor grade, or EOC. ROC curves, which plot the true positives (sensitivity) against the false positives (1-specificity) at different cutoff values, were used to assess the ability of particular logistic regression models to discern EOC cases from healthy women. The ROC summary statistic called the AUC, which estimates the probability of correctly discerning a person with cancer from a healthy person, was used to compare models (41). The AUC ranges from 0.5 (no discriminatory ability) to 1.0 (perfect discriminatory ability). Finally, test specificity and sensitivity were calculated for different cutoff values for sEGFR concentration and used to evaluate whether sEGFR is a potentially useful serum biomarker for screening and/or diagnosing EOC. Sensitivity and specificity refer to the probability of testing positive if disease is truly present and the probability of testing negative if disease is truly absent, respectively.

Results

Serum p110 sEGFR Is the Product of the 3-kb *EGFR/ERBB1* Alternate Transcript. Fig. 1 illustrates the protein structure of human p170 EGFR and p110 sEGFR as predicted from the full-length 5.8-kb and alternate 3.0-kb *EGFR/ERBB1* mRNA transcripts, respectively (31, 42). sEGFR is identical to EGFR until amino acid 603 according to the nucleotide numbering system of Ullrich *et al.* (42), and, hence, comprises extracellular subdomains I-IV (Fig. 1*A*). The first 603 amino acids of p110 sEGFR are followed by 78 unique COOH-terminal amino acids. To determine whether serum p110 sEGFR is encoded by the 3-kb *EGFR* transcript, we partially purified this protein by sequential passage of proteins through MAb affinity and anion exchange chromatography columns. Elution fractions from the anion exchange column enriched in p110 sEGFR were pooled, concentrated by ultrafiltration, and resolved by SDS-PAGE (data not shown). Serum p110 sEGFR was excised from a preparative SDS-PAGE gel and enzymatically digested with trypsin; the resulting tryptic peptides were then analyzed by Matrix Assisted Laser Desorption/Ionization-Time of Flight mass spectrometry. We observe that 18 of 43 tryptic peptides identified share homology with the extracellular domain of human EGFR (Fig. 1*B*). An additional peptide (#19) with a mass of 1029.56 Da shares complete identity with amino acids AMLFCLFK within the unique 78 amino acid COOH-terminal domain of the protein product predicted by the 3-kb *EGFR* transcript (Fig. 1*C*).

Serum p110 sEGFR Concentrations Are Significantly Lower in Patients with Early-Stage or Late-Stage Epithelial Ovarian Cancer Than in Healthy Women. To additionally explore the potential utility of serum p110 sEGFR concentrations as biomarkers of EOC, we compared sEGFR concentrations between 144 nonsurgical healthy female volunteers and 225 women with stage I through IV EOC who provided blood samples before cytoreductive surgery. The healthy women ranged in age from 20 to 76 years (median: 46 years); 81 women were classified as premenopausal, 3 as perimenopausal, 59 as postmenopausal, and 1 as indeterminate. The EOC patients ranged in age from 24 to 87 years (median: 61 years); 35 cases were classified as premenopausal, 183 as postmenopausal, and 7 as indeterminate. Thus, the EOC cases were comprised of 16% premenopausal and 81% postmenopausal women, whereas the controls were comprised of 56% premenopausal and 41% postmenopausal women (Table 1); 3% of both the cases and controls were perimenopausal or of indeterminate menopausal status. Overall, the cases were older than the controls for both the premenopausal (median age: 44 *versus* 32 years) and postmenopausal (median age: 63 *versus* 59 years) groups of women. Of the cases, 31 (14%), 13 (6%), 145 (64%),

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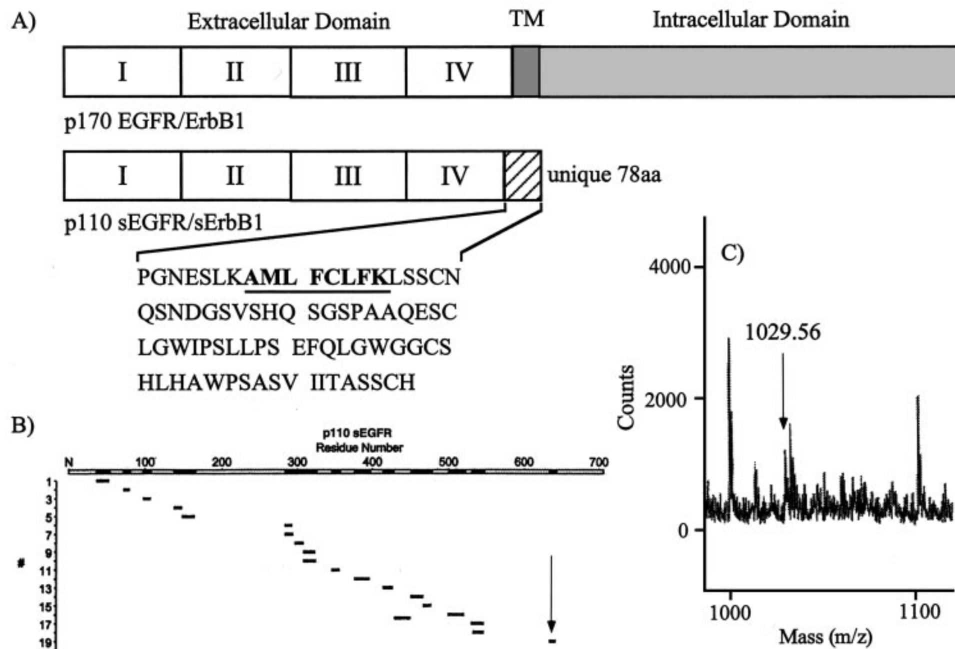

Fig. 1. *A,* this diagram compares the protein structure of the human p170 EGFR and p110 sEGFR as predicted from the full-length 5.8-kb and alternate 3.0-kb *EGFR/ERBB1* mRNA transcripts, respectively. The NH_2-terminal signal peptide is not shown in this diagram. p110 sEGFR consists of extracellular subdomains I-IV and is identical to EGFR until amino acid 603; the nucleotide numbering system is that of Ullrich *et al.* (42). The first 603 amino acids are followed by 78 unique COOH-terminal amino acids. *B,* the location of 19 trypsin-digested peptides of serum p110 sEGFR identified by mass spectrometry is shown on this linear map. This map includes the NH_2-terminal signal peptide. *C,* a portion of the mass spectrogram of the trypsin-digested peptides of p110 sEGFR is shown. The *arrow* at peak 1029.56 *m/z* represents peptide #19 (*arrow* in *B*) of serum p110 sEGFR with the following amino acid sequence: AMLFCLFK (*underlined* in *A*).

Table 1 Comparison of serum p110 sEGFR concentrations among healthy women and EOC cases, EOC cases with different stages of disease, and EOC cases with differ tumor grades

Patient groups	Median sEGFR (fmol/ml)	Range sEGFR (fmol/ml)	Wilcoxon *P*			
Healthy women (n = 144)[a]	7,177	114–31,465	<0.0001			
EOC cases (n = 225)[a]	463	ND[b]–82,436				
Premenopause						
Healthy women (n = 81; 56%)	8,835	341–24,294	<0.0001	<0.001	0.945	
EOC cases (n = 35; 16%)	417	ND–63,896				
Postmenopause						
Healthy women (n = 59; 41%)	3,400	114–31,465	<0.0001			
EOC cases (n = 183; 81%)	509	ND–82,436				
Healthy women (n = 41; 28%)[c]	5,300	114–31,465	<0.0001			
Stage I/II EOC (n = 41; 18%)[c]	1,031	ND–5,879				
Healthy women (n = 78; 54%)[c]	3,841	114–31,465	<0.0001			
Stage III/IV EOC (n = 78; 35%)[c]	356	ND–63,896				
Stage I/II EOC (n = 44; 20%)	1,084	ND–5,879	0.024			
Stage III/IV EOC (n = 180; 80%)	386	ND–82,436				
Stage I EOC (n = 31; 14%)	1,188	ND–5,879	0.625	0.009	0.963	0.464
Stage II EOC (n = 13; 6%)	1,018	ND–4,800	0.379			
Stage III EOC (n = 145; 64%)	343	ND–63,896	0.191			
Stage IV EOC (n = 35; 16%)	483	ND–82,436				
Grade I EOC (n = 23; 11%)	1,257	ND–8,130	0.008	0.023		
Grade II EOC (n = 36; 18%)	285	ND–7,138	0.257			
Grade III EOC (n = 145; 71%)	463	ND–82,436				

[a] 3% of both healthy women and EOC cases were of perimenopausal or indeterminate menopausal status.
[b] ND, nondetectable values below the interassay biological detection limit of 7.5 fmol/ml.
[c] Healthy women were matched randomly, one-to-one, on the basis of menopausal status and age (±2 years) to cases with stage I/II or stage III/IV EOC.

and 35 (16%) had stage I, II, III, and IV EOC, respectively; information concerning stage was not available for 1 EOC patient. The cases contained the following distribution of tumor histological subtypes: 109 papillary serous, 41 serous, 17 endometrioid, 18 primary peritoneal, 14 mixed, 4 clear cell, 3 mucinous, and 1 transitional cell. Finally, the cases were comprised of 23 (11%), 36 (18%), and 145 (71%) grade I, II, and III tumors, respectively. Histological subtype and grade were unavailable for 18 and 21 tumors, respectively. In general, the younger premenopausal group of EOC cases consisted of a proportionately greater number of early stage I/II (40% *versus* 16%) and low-grade I (36% *versus* 6%) tumors than the older

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postmenopausal group of EOC cases. Accordingly, early-stage I/II EOC cases (46%) comprised a proportionately greater number of low-grade I tumors than late stage III/IV EOC cases (2%), whereas late-stage III/IV EOC cases (98%) comprised a proportionately greater number of high-grade II/III tumors than early-stage I/II EOC cases (54%).

Overall, healthy women have significantly higher serum sEGFR concentrations than women with EOC (Fig. 2*A*; Table 1; Wilcoxon rank-sum test, $P < 0.0001$). After stratification based on menopausal status, we observe that serum sEGFR concentrations are significantly higher in healthy premenopausal than postmenopausal women (Wilcoxon rank-sum test, $P < 0.0001$) but do not differ between premenopausal and postmenopausal EOC patients (Wilcoxon rank-sum test, $P = 0.945$). We also note that serum sEGFR concentrations in EOC patients are significantly lower than in healthy women of identical menopausal status (Wilcoxon rank-sum test, $P < 0.0001$ for both comparisons). Comparison of sEGFR concentrations *versus* age shows that sEGFR concentrations decrease with age in healthy women (Fig. 3*A*; rho = −0.3411; $P < 0.0001$) but not in EOC cases (Fig. 3*B*; rho = 0.0133; $P = 0.843$).

To assess whether serum p110 sEGFR concentrations differ between healthy women and EOC cases with either early- or late-stage disease, we compared stage I/II or stage III/IV EOC cases to matched controls (Fig. 2*B*; Table 1). To account for the relationship of sEGFR concentrations and age or menopausal status in healthy women, EOC cases with stage I/II or stage III/IV disease were matched randomly, one-to-one, on the basis of menopausal status and age (±2 years) to healthy women. Comparison of cases to matched controls show that serum sEGFR concentrations are significantly lower in patients with EOC than healthy women for both stage I/II and stage III/IV disease (Wilcoxon signed-rank test, $P < 0.0001$ for both comparisons).

Serum p110 sEGFR Concentrations Are Not Associated with Disease Stage or Tumor Grade. To assess whether serum p110 sEGFR concentrations are associated with known prognostic factors of EOC, we compared EOC cases after stratification based on disease stage or tumor grade. Although we attempted to compare serum sEGFR concentrations between tumors of different histological subtypes, we did not have a sufficient number of nonserous tumors to make meaningful statistical comparisons (data not shown).

Comparison of stage I/II *versus* stage III/IV EOC shows that serum sEGFR concentrations are moderately lower in patients with late-stage EOC (Fig. 2C; Table 1; Wilcoxon rank-sum test, $P = 0.024$). Pair-wise comparisons of stage I through IV EOC show that sEGFR concentrations are significantly lower in patients with stage III *versus* stage I disease (Wilcoxon rank-sum test, $P = 0.009$); however, all of the other pair-wise comparisons do not differ significantly (Wilcoxon rank-sum test, $P \geq 0.191$). Because of the associations between grade and stage (rho = 0.3741; $P < 0.0001$), age and stage (rho = 0.2279; $P = 0.0006$), and menopausal status and stage (rho = 0.2017; $P = 0.0024$) among cases, we performed multivariate logistic regression analyses comparing stage I/II *versus* stage III/IV EOC to assess whether tumor grade, age, or menopausal status confound or modify the association between sEGFR concentration and disease stage (Table 2). Logistic regression analysis demonstrates that the association between log sEGFR concentration and EOC stage is of borderline significance (Wald χ^2, $P = 0.0770$), such that the odds of a one log unit decrease in sEGFR concentration is 1.148 (95% CI, 0.99–1.3) times greater for stage III/IV *versus* stage I/II EOC. After

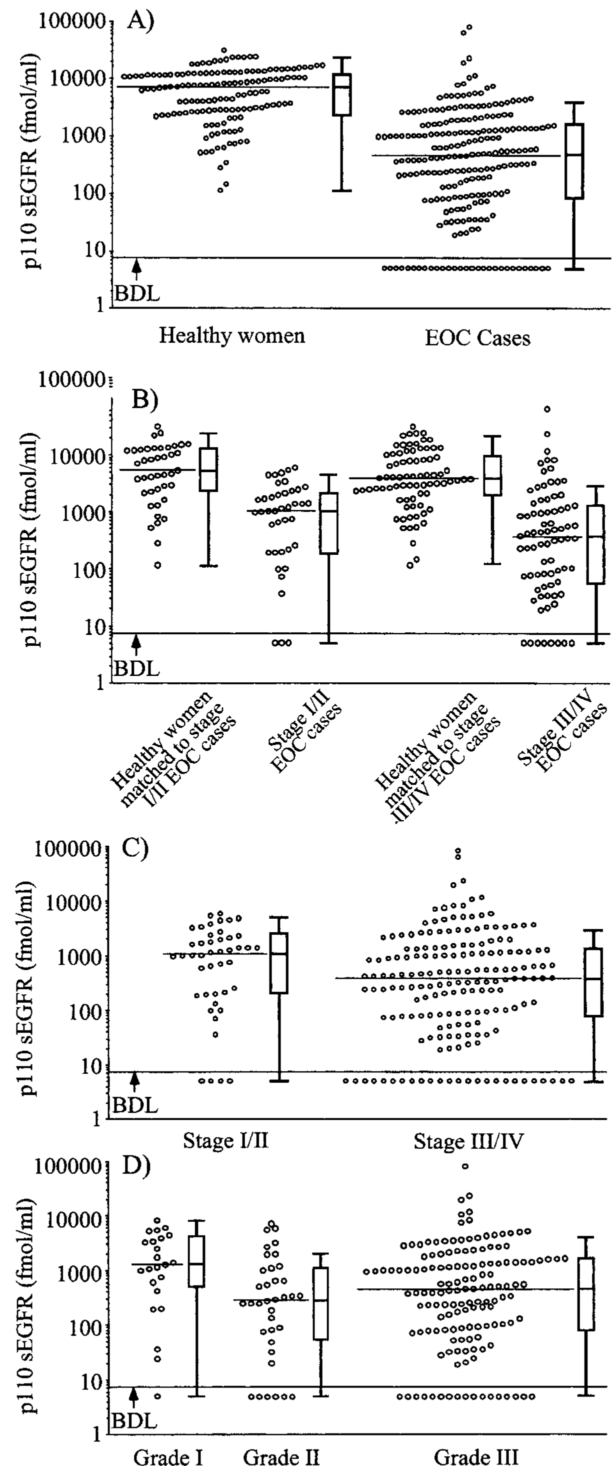

Fig. 2. Serum p110 sEGFR concentrations between healthy female volunteers and cases with stage I-IV EOC (*A*), healthy female volunteers, matched one-to-one on the basis of age and menopausal status, and cases with either stage I/II or stage III/IV EOC (*B*), cases with stage I/II and stage III/IV EOC (*C*), and cases with grade I, grade II, and grade III tumors (*D*) are compared in these scattergrams. Each *data point* represents the median sEGFR concentration for one serum sample assayed three times in duplicate. The *horizontal lines* indicate the median serum p110 sEGFR concentration for these groups of healthy females and cases with EOC. *Horizontal lines in the box plot* represent the first, second (median), and third quartiles; *whiskers* extend from the box to a distance of 1.5 interquartile ranges. The interassay BDL for the ALISAs performed in this study is marked as shown (*horizontal line with arrow*); *bars,* ±SD.

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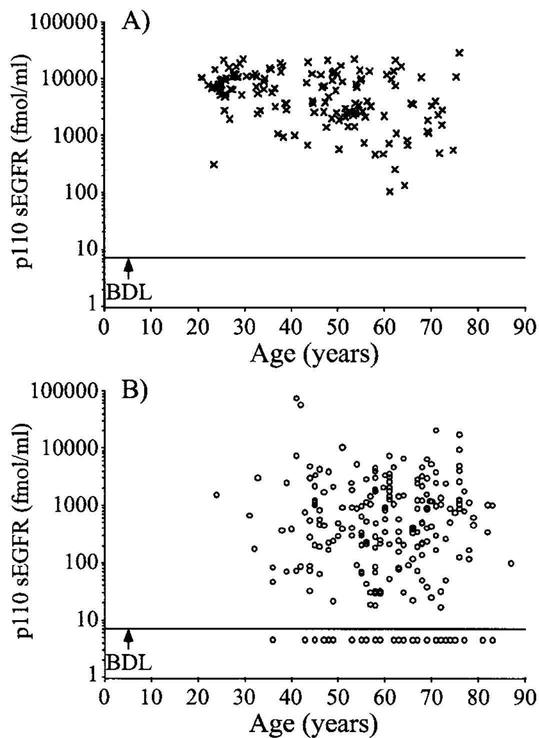

Fig. 3. Serum p110 sEGFR concentrations in healthy women (*A*) and EOC patients (*B*) are plotted against age. Each *data point* represents the median sEGFR concentration for one serum sample assayed three times in duplicate. The inter-assay BDL for the ALISAs performed in this study is marked as shown (*horizontal line with arrow*).

independently adjusting for tumor grade, age on a continuous scale, or menopausal status we observe that the odds of a one log unit decrease in sEGFR concentration is 1.125 (95% CI, 0.95–1.3), 1.155 (95% CI, 0.98–1.4), and 1.167 (95% CI, 0.99–1.4) times greater for stage III/IV *versus* stage I/II EOC, respectively. After simultaneously adjusting for grade plus age or grade plus menopausal status, the odds of a one log unit decrease in sEGFR concentration is 1.122 (95% CI, 0.94–1.3) and 1.133 (95% CI, 0.95–1.3) times greater for stage III/IV *versus* stage I/II EOC, respectively. Therefore, multivariate models show that tumor grade, age, menopausal status, and grade plus age or menopausal status confound the association between sEGFR concentration and disease stage. Altogether, these analyses show that sEGFR concentrations do not differ between cases of stage I/II and stage III/IV EOC, after adjusting for tumor grade (Wald χ^2, $P = 0.1728$), age (Wald χ^2, $P = 0.0803$), menopausal status (Wald χ^2, $P = 0.0596$), grade plus age (Wald χ^2, $P = 0.1943$), or grade plus menopausal status (Wald χ^2, $P = 0.1589$).

Pair-wise comparisons of sEGFR concentrations in patients with grade I, II, and III tumors show that sEGFR concentrations are lower in patients with grade II (Wilcoxon rank-sum test, $P = 0.008$) or grade III (Wilcoxon rank-sum test, $P = 0.023$) tumors compared with grade I tumors (Fig. 2*D*: Table 1). Univariate logistic regression demonstrates an association of borderline significance between log sEGFR concentration and tumor grade (Wald χ^2, $P = 0.0631$), such that the odds of a one log unit decrease in sEGFR concentration is 1.228 (95% CI, 0.99–1.5) times greater for grade II/III *versus* grade I tumors (Table 2). After independently adjusting for age on a continuous scale, menopausal status, or disease stage in multivariate models, we observe that the odds of a one log unit decrease in sEGFR concentration is 1.254 (95% CI, 0.99–1.6), 1.292 (95% CI, 1.01–1.6), and 1.184 (95% CI, 0.89–1.6) times greater for grade II/III *versus* grade I tumors, respectively. Thus, log sEGFR concentrations appear to be associated with tumor grade after accounting for age (Wald χ^2, $P = 0.0626$; borderline significance) or menopausal status (Wald χ^2, $P = 0.0384$). However, after simultaneously adjusting for stage plus age or menopausal status, we observe that the odds of a one log unit decrease in sEGFR concentration is 1.26 (95% CI, 0.93–1.7) and 1.341 (95% CI, 0.95–1.9) times greater for grade II/III *versus* grade I tumors, respectively. Therefore, disease stage, stage plus age, and stage plus menopausal status confound the association between sEGFR concentration and tumor grade. Taken together, these analyses show that sEGFR concentrations do not differ between low-grade I and higher-grade II/III tumors, after adjusting for disease stage independently (Wald χ^2, $P = 0.2479$), or together with age (Wald χ^2, $P = 0.1416$) or menopausal status (Wald χ^2, $P = 0.0917$).

Low Serum p110 sEGFR Concentrations as a Risk Factor and Screening Biomarker for Epithelial Ovarian Cancer. Dynamic temporal changes in serum biomarker concentrations cannot be inferred from a single preoperative blood sample taken from an individual; therefore, we do not know whether serum p110 sEGFR concentrations decreased before or after the initiation of epithelial ovarian tumor growth, or whether certain individuals simply have an inherently low level of serum sEGFR. Despite this caveat, and because serum sEGFR concentrations are not related to tumor burden (disease stage), we used logistic regression models to evaluate serum sEGFR concentrations as a possible risk factor for EOC (Table 3). Dichotomization of serum sEGFR concentrations into high values (>median sEGFR concentration of both healthy women and EOC patients combined) and low values (≤median) reveals that women with low sEGFR concentrations have a significantly greater risk of EOC than women with high sEGFR concentrations (OR, 16.4; 95% CI, 9.4–28.8). Additional stratification of sEGFR concentrations into quartiles reveals that the association between sEGFR concentrations and EOC is characterized by a dose-response relationship with a significant linear component (P for trend < 0.0001). These analyses show that women with EOC have a 7.1 (95% CI, 3.4–14.9), 26.0 (95% CI, 11.7–57.7), and 282.6 (95% CI, 61.3–1302.1) higher odds of having sEGFR concentrations between 1,404 and 5,731 fmol/ml (3rd quartile), 273 and 1403 fmol/ml (2nd quartile), or nondetectable and 272 fmol/ml (1st quartile) compared with healthy women with sEGFR concentrations between 5,732 and 82,436 fmol/ml (4th quartile), respectively. Multivariate models of dichotomized sEGFR concentrations demonstrate a significant interaction between sEGFR concentration and age as a continuous variable (Wald χ^2, $P = 0.0223$ for age), as well as between sEGFR concentration and menopausal status (Wald χ^2, $P = 0.007$ for menopausal status). The logistic regression model, which includes p110 sEGFR as a dichotomous variable, menopausal status, and p110 sEGFR as a dichotomous variable × menopausal status, shows that postmenopausal women with high serum sEGFR concentrations (OR, 3.3; 95% CI, 2.2–5.0), postmenopausal women with low serum sEGFR concentrations (OR, 25.4; 95% CI, 1.3–507.2), and premenopausal women with low serum sEGFR concentrations (OR, 51.3; 95% CI, 15.4–170.4) have a significantly greater risk of EOC than premenopausal women with high serum sEGFR concentrations.

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Table 2 ORs, 95% CIs, and percentage of confounding (%) for a 1 log unit decrease in serum p110 sEGFR concentration among EOC cases with stage III/IV *versus* stage I/II disease or grade II/III *versus* grade I tumors

	Stage III/IV *versus* stage I/II OR (CI) %	Wald χ^2 P^a	Grade II/III *versus* grade I OR (CI) %	Wald χ^2 P^a
Crude	1.148 (0.99, 1.3)	0.0770	1.228 (0.99, 1.5)	0.0631
Grade-adjusted	1.125 (0.95, 1.3) 14.7%	0.1728		
Age-adjusted	1.155 (0.98, 1.4) −4.3%	0.0803	1.254 (0.99, 1.6) −9.9%	0.0626
Menopause-adjusted	1.167 (0.99, 1.4) −11.3%	0.0596	1.292 (1.01, 1.6) −24.5%	0.0384
Grade- and age-adjusted	1.122 (0.94, 1.3) 16.8%	0.1943		
Grade- and menopause-adjusted	1.133 (0.95, 1.3) 9.9%	0.1589		
Stage-adjusted			1.184 (0.89, 1.6) 18.0%	0.2479
Stage- and age-adjusted			1.260 (0.93, 1.7) −12.5%	0.1416
Stage- and menopause-adjusted			1.341 (0.95, 1.9) −42.5%	0.0917

[a] Wald χ^2 P for log (p110 sEGFR) in each logistic regression model.

Table 3 ORs and 95% CIs comparing p110 sEGFR concentrations between EOC cases and healthy women

p110 sEGFR concentration	OR (CI)
Low (≤median; 1,403 fmol/ml)	16.4 (9.4, 28.8)
High (>median; referent)	1.0
P for trend	<0.0001
1st quartile (ND[a]–272 fmol/ml)	282.6 (61.3, 1302.1)
2nd quartile (273–1,403 fmol/ml)	26.0 (11.7, 57.7)
3rd quartile (1,403–5,731 fmol/ml)	7.1 (3.4, 14.9)
4th quartile (5,732–82,436 fmol/ml; referent)	1.0
P for trend	<0.0001
Low (≤median; postmenopausal women)	25.4 (1.3, 507.2)
Low (≤median; premenopausal women)	51.3 (15.4, 170.4)
High (>median; postmenopausal women)	3.3 (2.2, 5.0)
High (>median; premenopausal women; referent)	1.0

[a] ND, nondetectable values below the interassay biological detection limit of 7.5 fmol/ml.

When sEGFR concentrations are evaluated on a continuous scale, logistic regression analyses show that the association of sEGFR concentration with EOC has a significant curvilinear component (data not shown in Table 3; Wald χ^2, $P < 0.0001$ for both sEGFR and sEGFR^2). Multivariate curvilinear models additionally show that this association is modified by age (Wald χ^2, $P = 0.0054$ for age), whereby older age reduces the risk of EOC for an incremental decrease in sEGFR concentration. For example, the full logistic regression model, which includes p110 sEGFR, p110 sEGFR^2, age, and p110 sEGFR × age as continuous variables, shows that 20, 30, or 40-year-old women with EOC have a 2.2 (95% CI, 1.3–3.6), 2.0 (95% CI, 1.2–3.5), and 1.9 (95% CI, 1.004–3.4) higher odds of a 1000 fmol/ml lower serum sEGFR concentration than healthy women of equal age, respectively. This regression model also shows that larger incremental decreases in sEGFR concentration are associated with greater risk of EOC. For example, women with EOC in their 20th year of life have a 1.08 (95% CI, 1.03–1.14), 2.2 (95% CI, 1.3–3.6), and 38.5 (95% CI, 2.9–512.4) higher odds of a 100 fmol/ml, 1000 fmol/ml, or 5000 fmol/ml lower serum sEGFR concentration than healthy 20-year-old women, respectively. Taken together, these analyses show that serum sEGFR concentrations may be a useful biomarker of EOC risk, particularly in young, premenopausal women.

To evaluate the potential screening utility of serum p110 sEGFR concentrations for EOC we generated ROC curves comparing healthy women to patients with EOC. To account for the interaction between sEGFR concentrations and age, as well as the curvilinear association between sEGFR concentra-

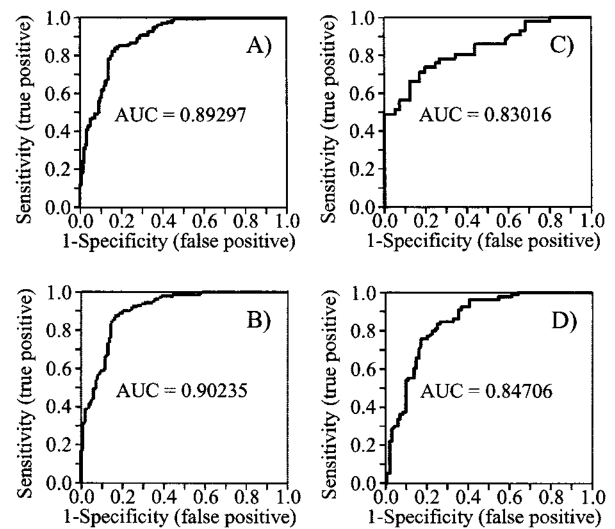

Fig. 4. ROC curves comparing p110 sEGFR concentrations in all healthy women *versus* all EOC cases (*A*), and all healthy women *versus* stage III/IV EOC cases (*B*) are shown for the full logistic regression model, which includes p110 sEGFR, p110 sEGFR^2, age, and p110 sEGFR × age as continuous variables. ROC curves comparing p110 sEGFR concentrations between age- and menopausal status-matched stage I/II EOC cases and healthy women (*C*), and age- and menopausal status-matched stage III/IV EOC cases and healthy women (*D*) are shown for the completely reduced model, which includes only p110 sEGFR as a continuous variable.

tion and EOC, ROC analyses were performed using the full logistic regression model, which includes the terms p110 sEGFR, p110 sEGFR^2, age, and p110 sEGFR × age as continuous variables. With this model, we observe an AUC of 0.89297 and 0.90235 for the comparison of all of the healthy women *versus* all of the EOC cases (Fig. 4*A*), and all of the healthy women *versus* all of the stage III/IV EOC cases (Fig. 4*B*), respectively. We were unable to assess the ability of the full model (or a reduced curvilinear model) to distinguish all of the stage I/II EOC cases from all of the healthy women; these models did not remain significant for all of the covariates, most likely, because of the small size of the stage I/II group of EOC cases. To assess the ability of sEGFR concentrations to discern early- or late-stage EOC cases from healthy women, we, therefore, performed ROC analyses with the age- and menopausal status-matched stage I/II or stage III/IV EOC cases *versus* healthy controls (see Table 1) using a completely reduced logistic regression model, which includes only p110 sEGFR as a continuous variable. With the reduced model, we observe an

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Table 4 Statistics of test validity for p110 sEGFR concentration

	All stages EOC	Stage I/II EOC	Stage III/IV EOC	Healthy
All women				
Test positive	125	15	110	8
Test negative	100	29	70	136
Total	225	44	180	144
Sensitivity (%)	55.6%	34.1%	61.1%	
Specificity (%)				94.4%
95% cut-off[a]				624 fmol/ml
Premenopausal women				
Test positive	26	9	17	5
Test negative	9	5	4	76
Total	35	14	21	81
Sensitivity (%)	74.3%	64.3%	81.0%	
Specificity (%)				93.8%
95% cut-off[a]				1185 fmol/ml
Postmenopausal women				
Test positive	91	8	83	3
Test negative	92	21	70	56
Total	183	29	153	59
Sensitivity (%)	49.7%	27.6%	54.2%	
Specificity (%)				94.9%
95% cut-off[a]				495 fmol/ml
Women ages 20–40 years				
Test positive	8	2	6	4
Test negative	3	1	2	59
Total	11	3	8	63
Sensitivity (%)	72.7%	66.7%	75.0%	
Specificity (%)				93.7%
95% cut-off[a]				1283 fmol/ml
Women ages 41–60 years				
Test positive	60	11	49	3
Test negative	39	16	23	51
Total	99	27	72	54
Sensitivity (%)	60.6%	40.7%	68.1%	
Specificity (%)				94.4%
95% cut-off[a]				707 fmol/ml
Women ages 61–87 years				
Test positive	38	4	34	2
Test negative	76	10	66	25
Total	114	14	100	27
Sensitivity (%)	33.3%	28.6%	34.0%	
Specificity (%)				92.6%
95% cut-off[a]				186 fmol/ml

[a] Cut-off value represents the 95% lower limit of sEGFR concentration in healthy women for each group.

AUC of 0.83016 and 0.84706 for the comparison of matched stage I/II EOC cases *versus* controls (Fig. 4*C*) and matched stage III/IV EOC cases *versus* controls (Fig. 4*D*), respectively. On the whole, these analyses indicate that sEGFR concentrations are somewhat more effective in discerning EOC cases with late-stage than early-stage disease from healthy women, and that sEGFR concentrations have an 89% probability (AUC = 0.89297) of correctly discerning EOC patients from healthy women when accounting for the interaction between sEGFR concentration and age.

Logistic regression analyses indicate that age- and menopausal status-specific cutoff values for sEGFR concentration are appropriate. Among healthy women, 95% of the p110 sEGFR values are equal to or above 624 fmol/ml; therefore, we chose 624 fmol/ml as the cutoff value and set test specificity equal to 95% (Table 4). For the comparison of all of the EOC patients to all of the healthy women, we observe a test sensitivity of 55.6%. In comparison, the sensitivity for stage I/II (46% grade I tumors) or stage III/IV (98% grade II/III tumors) EOC is 34.1% and 61.1%, respectively. After stratifying the cases and controls into groups based on menopausal status and selecting appropriate cutoff values to maintain a specificity of ~95% across strata, we observe a sensitivity of 74.3% and 49.7% for premenopausal *versus* postmenopausal women, respectively. The test sensitivity for detecting stage I/II or stage III/IV EOC from healthy premenopausal *versus* postmenopausal women is 64.3% or 81.0%, and 27.6% or 54.2%, respectively. Stratification of the cases and controls into groups between 20–40, 41–60, and 61–87 years of age, followed by selection of cutoff values at the lower 95th percentile for each group of controls show a sensitivity of 72.7, 60.6, and 33.3% for each age group, respectively. The test sensitivity for detecting stage I/II or stage III/IV EOC from healthy women in these age groups is 66.7% or 75.0%, 40.7% or 68.1%, and 28.6% or 34.0%, respectively. By maintaining a specificity of ~95% across groups with menopausal status- or age-specific cutoff values, these analyses indicate that as a screening test for EOC, sEGFR concentrations are most useful for detecting EOC among young, premenopausal women.

Discussion

Estrogen is known to regulate *ERBB* gene expression (43–47). In this regard, we have shown recently that p110 sEGFR concentrations exhibit an age-gender interaction, increasing with age in men but decreasing with age in women (34). Consequently, sEGFR concentrations are higher in premenopausal women compared either with postmenopausal women or with age-matched men. Furthermore, we have shown that in healthy women, serum sEGFR concentrations have negative associations with both follicle-stimulating hormone and luteinizing hormone concentrations, suggesting that gonadotropic hormones may modulate the expression of sEGFR. Therefore, we propose that circulating steroid and gonadotropic hormones may be involved in regulating the biosynthesis and/or release of sEGFR into circulatory fluids. Importantly, these observations indicate that age, menstrual cycle phase, menopausal status, and exogenous hormone use, in the form of oral contraceptives or hormone replacement therapy, may be potential confounders or effect modifiers of the association of serum sEGFR concentrations and human cancer. Accordingly, it may be necessary to take these variables into account when using sEGFR concentrations in risk assessment, screening, and/or diagnosis of cancer.

Limitations and caveats of this study include: (*a*) insufficient sample size to assess potential differences in serum p110 sEGFR concentrations among histological subtypes of EOC; (*b*) lack of data on potential confounders or effect modifiers of serum sEGFR concentrations such as family history, *BRCA1/2* mutation status, menstrual cycle phase, parity, and exogenous hormone use; (*c*) selection bias of the control group, which was not selected at random from the same source population as the cases; and (*d*) an inability to assess temporally the risk of developing EOC among asymptomatic women with prediagnostic serum samples. Given these caveats and limitations, a well-designed prospective nested case-control study of EOC will be needed to confirm that serum sEGFR concentration may be a useful risk factor, screening, and/or perhaps diagnostic biomarker of EOC. Such a study should include incident cases with EOC, benign ovarian neoplasms, and other benign gynecologic conditions that require surgery, as well as healthy women selected randomly from the same source population. Nonetheless, the observations reported in this study are con-

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sistent with the hypothesis that serum sEGFR may have clinical utility as a new biomarker of EOC.

Clinicopathological and epidemiological prognosticators such as advanced surgical stage, high tumor grade, large residual volume of tumor after cytoreductive surgery, advanced age, and lower performance status have been associated previously with poor patient prognosis for EOC (48, 49). Accordingly, selection of postsurgical chemotherapy for EOC is based currently on knowledge of tumor histology and grade, surgical stage, and residual disease after surgery. However, in the future it is likely that therapeutic decisions will be based on the expression of a combination of tumor and/or serum biomarkers. In this regard, preoperative serum p105 sErbB2 concentrations have been found to be elevated in women with ovarian cancer in comparison with healthy women (50–53). In a study consisting of 57 incident EOC cases, elevated preoperative serum sErbB2 concentrations were not associated with disease stage, tumor grade, histological subtype, or serum CA-125 concentrations (51). Nevertheless, high preoperative serum sErbB2 concentrations were significantly associated with shorter survival time for these surgically treated cases of EOC suggesting that serum sErbB2 concentrations may be independent prognosticators. Here, we confirm our previous finding that preoperative serum sEGFR concentrations are significantly lower in stage III/IV EOC patients than in healthy females (33). We also report that serum sEGFR concentrations are significantly lower in stage I/II EOC patients than in healthy women, even after accounting for effect modification by age or menopausal status. Furthermore, we demonstrate that preoperative serum p110 sEGFR concentrations are not associated with disease stage or tumor grade, after reciprocally adjusting for either tumor grade or disease stage alone or in combination with either age or menopausal status, respectively. Although the relationship between serum p110 sEGFR concentration and tumor EGFR expression is currently unknown, a positive immunohistochemical status of EGFR has been reported recently to be an independent prognostic factor of EOC, thus highlighting the potential importance of circulating sEGFR isoforms as prognostic factors for this disease (54). We surmise that additional clinical studies are warranted to evaluate whether serum sEGFR concentrations can be used independently to predict the prognosis of ovarian cancer patients.

The goal of cancer risk assessment and screening is to detect precancerous lesions or early-stage malignancies in the asymptomatic preclinical phase of disease, such that subsequent diagnosis and treatment will have a significant impact on reducing disease morbidity and mortality (55). Although dietary factors, environmental exposures, female reproductive characteristics, and various medical conditions have been weakly to moderately associated with a higher risk of EOC (56–60), there are no definitive risk factors that lend themselves to prevention and early detection strategies for the 90% of epithelial ovarian cancers that develop sporadically (6, 7). Fortunately, serum biomarkers may provide a useful and cost-effective tool for the risk assessment of EOC (9). To assess the association of serum CA-125 values and EOC risk, Helzlsouer *et al.* (61) performed a nested case-control study among a cohort of 11,009 women who donated blood to a community-based serum bank. Among women of the same age and menopausal status, serum CA-125 values >10 units/ml were found to be associated with a 3.4-fold (95% CI, 1.3–8.8) greater relative risk of developing EOC within the next 15 years. Moreover, the relative risk of developing EOC increased with higher serum CA-125 values; CA-125 values >35 units/ml were associated with a relative risk of 15.1 (95% CI, 1.9–120.8). In a cohort study of 22,000 asymptomatic postmenopausal women, Jacobs *et al.* (62) more recently reported that the relative risk of EOC is 36-fold (95% CI, 18.3–70.4) and 205-fold (95% CI, 79.0–530.7) greater in women with serum CA-125 values ≥30 units/ml and ≥100 units/ml after 1 year of follow-up, respectively. Subsequent pelvic ultrasonography of women with elevated CA-125 levels (≥30 units/ml) identified women with abnormal ovarian morphology who had a 327-fold increased relative risk of developing index cancers, which were defined as primary invasive epithelial carcinomas of the ovary and fallopian tube, after 1 year of follow-up compared with women with normal ovarian morphology (63). Importantly, women with elevated CA-125 values and normal ovarian morphology were at no more risk of index cancers than the entire study population. In the case-control study presented here, we show that women with low preoperative serum p110 sEGFR concentrations have a greater risk of EOC than women with high sEGFR concentrations. Moreover, we observe an interaction between age or menopausal status and serum sEGFR concentrations, whereby older age or menopause decreases the odds that an incrementally lower sEGFR concentration is associated with EOC. These data indicate that serum sEGFR may be more effective in assessing the risk of EOC in younger premenopausal women than in older postmenopausal women. We suggest that serum sEGFR, like CA-125, may be useful for assessing the risk of EOC in the general population and, in particular, for stratifying asymptomatic premenopausal women into high-risk subgroups for additional evaluation. We envision that the combined use of sEGFR, CA-125, other serum biomarkers, and ultrasonography may be effective in assigning EOC risk to individual women.

Developing a test as a screening and/or diagnostic tool for cancer involves the evaluation of a number of statistics including sensitivity, specificity, and AUC (64, 65). Although novel serum biomarkers (66–71) and novel technologies to identify EOC-specific serological proteomic patterns (72) are under investigation as potential screening tests for EOC, CA-125 remains the standard serum biomarker for comparison (73, 74). Initial reports from retrospective, nested case-control studies documented specificities for CA-125 ranging from 92.6% to 95.4% (39, 75). In a prospective cohort study of 5550 women, Einhorn *et al.* (76) identified 175 women with elevated CA-125 values (≥30 units/ml) along with an equal number of age-matched controls with low CA-125 values. This study reported a specificity of 97% for EOC patients >50 years of age and 91% for EOC patients ≤50 years of age for the sequential use of CA-125 (cutoff value of 30 units/ml) followed by pelvic examination and transabdominal sonography as a screening modality. Used as a primary screening test, CA-125 is, thus, more specific in older postmenopausal patients. In a nested case-control study of 110 EOC patients, Helzlsouer *et al.* (61) subsequently reported a sensitivity of 57% and a specificity of 100% with a cutoff value of 35 units/ml CA-125. These investigators and others (77) concluded that, although serum CA-125 values alone are highly specific, they are insufficiently sensitive to recommend population-based screening for EOC. A pooled analysis of CA-125 values of >35 units/ml among all of the published studies calculated sensitivities of 51, 71, 91, and 98% for stage I, II, III, and IV EOC, respectively, and 85% for all of the stages combined (73). Here, we show that serum p110 sEGFR concentrations have an 89% probability (AUC = 0.89297) of correctly discerning an EOC patient from a healthy woman using ROC analyses with a full logistic regression model that accounts for the effect of age on the association between sEGFR and EOC. Logistic regression and ROC analyses also indicate that age- and menopausal status-specific cutoff values for sEGFR concentration are appropriate, and that sEGFR concentrations are more effective in discerning

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stage III/IV than stage I/II disease from healthy women. Using a cutoff value that sets specificity at ~95%, sEGFR concentrations have a sensitivity of 34.1, 61.1, and 55.6% for detecting stage I/II (46% grade I tumors), stage III/IV (98% grade II/III tumors), and all of the stages of EOC, respectively. After selecting cutoff values that maintain ~95% specificity across strata of menopausal status or age, we observe that sEGFR concentrations are most useful for detecting stage III/IV disease among young, premenopausal women. Among women between 20 and 40 years of age we observe sensitivities of 66.7% and 75.0% for stage I/II or stage III/IV EOC, respectively, and 72.7% for all of the stages combined. Among premenopausal women we observe sensitivities of 64.3% and 81.0% for stage I/II or stage III/IV EOC, respectively, and 74.3% for all of the stages combined. Accordingly, we suggest that serum sEGFR may have application as a screening and/or diagnostic test for EOC, particularly when used in conjunction with other tumor biomarkers and sonographic imaging modalities among young, premenopausal women.

In conclusion, we have identified circulating p110 sEGFR as the product of a 3-kb alternate transcript of the human *EGFR/ERBB1* gene. Additionally, we have shown that the concentration of the soluble EGFR isoform is lower in women with both early- and advanced-stage EOC. These results are consistent with the hypothesis that sEGFR may have clinical utility as a novel serum biomarker of EOC. We conclude that serum sEGFR warrants additional investigation for the risk assessment, screening, and/or diagnosis of EOC.

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References

1. Jemal, A., Thomas, A., Murray, T., and Thun, M. Cancer statistics, 2002. CA Cancer J. Clin., *52:* 23–47, 2002.

2. Berek, J. S., and Bast, R. C., Jr. Ovarian cancer screening. The use of serial complementary tumor markers to improve sensitivity and specificity for early detection. Cancer (Phila.), *76:* 2092–2096, 1995.

3. Karlan, B. Y. Screening for ovarian cancer: what are the optimal surrogate endpoints for clinical trials? J. Cell. Biochem., *23*(Suppl.)*:* 227–232, 1995.

4. Ozols, R. F., Rubin, S. C., Thomas, G., and Robboy, S. Epithelial ovarian cancer. *In:* W. J. Hoskins, C. A. Perez, and R. C. Young (eds.), Principles and Practice of Gynecol. Oncol., pp. 919–986. Philadelphia, PA: Lippincott-Raven Publishers, 1997.

5. Goff, B. A., Mandel, L., Muntz, H. G., and Melancon, C. H. Ovarian carcinoma diagnosis. Cancer (Phila.), *89:* 2068–2075, 2000.

6. La Vecchia, C. Epidemiology of ovarian cancer: a summary review. Eur. J. Cancer Prev., *10:* 125–129, 2001.

7. Runnebaum, I. B., and Stickeler, E. Epidemiological and molecular aspects of ovarian cancer risk. J. Cancer Res. Clin. Oncol., *127:* 73–79, 2001.

8. Young, R. C., Walton, L. A., Ellenberg, S. S., Homesley, H. D., Wilbanks, G. D., Decker, D. G., Miller, A., Park, R., and Major, F., Jr. Adjuvant therapy in stage I and stage II epithelial ovarian cancer. Results of two prospective randomized trials. N. Engl. J. Med., *322:* 1021–1027, 1990.

9. Urban, N. Screening for ovarian cancer. We now need a definitive randomised trial. Br. Med. J., *319:* 1317–1318, 1999.

10. Young, R. C., Decker, D. G., Wharton, J. T., Piver, M. S., Sindelar, W. F., Edwards, B. K., and Smith, J. P. Staging laparotomy in early ovarian cancer. JAMA, *250:* 3072–3076, 1983.

11. McGowan, L. Patterns of care in carcinoma of the ovary. Cancer (Phila.), *71:* 628–633, 1993.

12. Srivastava, S., and Kramer, B. S. Early detection cancer research network. Lab. Investig., *80:* 1147–1148, 2000.

13. Fishman, D. A., Cohen, L., Bozorgi, K., Singh, D., O'Donnell, A., Donnelly, M., O'Rourke, J., Pfeifer, K., Lu, T., Maihle, N. J., Baron, A. T., Stack, M. S., Lurain, J., and Schwartz, P. E. The scientific basis for the accurate detection of early stage epithelial ovarian carcinoma, part 1. The Female Patient, *27:* 11–15, 2002.

14. Brandt-Rauf, P. W. The c-erbB transmembrane growth factor receptors as serum biomarkers in human cancer studies. Mutat. Res., *333:* 203–208, 1995.

15. McKenzie, S. J. Diagnostic utility of oncogenes and their products in human cancer. Biochim. Biophys. Acta, *1072:* 193–214, 1991.

16. Lafky, J. M., Greenewood, T. M., Baron, A. T., Boardman, C. H., Cora, E. M., and Maihle, N. J. Soluble ErbB receptors (sEGFR/sErbBs): serum biomarker in breast and ovarian cancer. *In:* E. P. Diamandis, H. A. Fritsche, H. Lilja, D. W. Chan, and M. K. Schwartz (eds.), Tumor Markers: Physiology, Pathobiology, Technology and Clinical Applications, pp. 427–431. Washington, D. C.: American Association for Clinical Chemistry, Inc., 2002.

17. Maihle, N. J., Baron, A. T., Barrette, B. A., Boardman, C. H., Christensen, T. A., Cora, E. M., Faupel-Badger, J. M., Greenewood, T., Juneja, S. C., Lafky, J. M., Lee, H., Reiter, J. L., and Podratz, K. C. EGF/ErbB receptor family in ovarian cancer. *In:* M. S. Stack and D. A. Fishman (eds.), Ovarian Cancer, pp. 247–258. Boston, MA: Kluwer Academic Publishers, 2002.

18. Zabrecky, J. R., Lam, T., McKenzie, S. J., and Carney, W. The extracellular domain of p185/*neu* is released from the surface of human breast carcinoma cells. SK-BR-3. J. Biol. Chem., *266:* 1716–1720, 1991.

19. Pupa, S. M., Menard, S., Morelli, D., Pozzi, B., De Palo, G., and Colnaghi, M. I. The extracellular domain of the c-*erbB*-2 oncoprotein is released from tumor cells by proteolytic cleavage. Oncogene, *8:* 2917–2923, 1993.

20. Petch, L. A., Harris, J., Raymond, V. W., Blasband, A., Lee, D. C., and Earp, H. S. A truncated, secreted form of the epidermal growth factor receptor is encoded by an alternatively spliced transcript in normal rat tissue. Mol. Cell. Biol., *10:* 2973–2982, 1990.

21. Ilekis, J., Stark, B. C., and Benveniste, R. Expression of epidermal growth factor receptors at the mRNA and cell membrane levels in first trimester and term human placentae. *In:* R. K. Miller and H. A. Thiede (eds.), Molecular Biology and Cell Regulation of the Placenta, Trophoblast Research 5, pp. 103–117. New York, NY: Plenum, 1991.

22. Ilekis, J. V., Stark, B. C., and Scoccia, B. Possible role of variant RNA transcripts in the regulation of epidermal growth factor receptor expression in human placenta. Mol. Reprod. Dev., *41:* 149–156, 1995.

23. Maihle, N. J., Flickinger, T. W., Raines, M. A., Sanders, M. L., and Kung, H-J. Native avian c-*erbB* gene expresses a secreted protein product corresponding to the ligand-binding domain of the receptor. Proc. Natl. Acad. Sci. USA, *88:* 1825–1829, 1991.

24. Katoh, M., Yazaki, Y., Sugimura, T., and Terada, M. c-*erb*B3 gene encodes secreted as well as transmembrane receptor tyrosine kinase. Biochem. Biophys. Res. Commun., *192:* 1189–1197, 1993.

25. Scott, G. K., Robles, R., Park, J. W., Montgomery, P. A., Daniel, J., Holmes, W. E., Lee, J., Keller, G. A., Li, W-L., Fendly, B. M., Wood, W. I., Shepard, H. M., and Benz, C. C. A truncated intracellular HER2/*neu* receptor produced by alternative RNA processing affects growth of human carcinoma cells. Mol. Cell. Biol., *13:* 2247–2257, 1993.

26. Das, S. K., Tsukamura, H., Paria, B. C., Andrews, G. K., and Dey, S. K. Differential expression of epidermal growth factor receptor (EGF-R) gene and regulation of EGF-R bioactivity by progesterone and estrogen in the adult mouse uterus. Endocrinology, *134:* 971–981, 1994.

27. Reiter, J. L., and Maihle, N. J. A 1.8 kb alternative transcript from the human epidermal growth factor receptor gene encodes a truncated form of the receptor. Nucleic Acids Res., *24:* 4050–4056, 1996.

28. Tong, B. J., Das, S. K., Threadgill, D., Magnuson, T., and Dey, S. K. Differential expression of the full-length and truncated forms of the epidermal growth factor receptor in the preimplantation mouse uterus and blastocyst. Endocrinology, *137:* 1492–1496, 1996.

29. Lee, H., and Maihle, N. J. Isolation and characterization of four alternate c-*erbB3* transcripts expressed in ovarian carcinoma derived cell lines and normal human tissues. Oncogene, *16:* 3243–3252, 1998.

30. Doherty, J. K., Bond, C., Jardim, A., Adelman, J. P., and Clinton, G. M. The *HER-2/neu* receptor tyrosine kinase gene encodes a secreted autoinhibitor. Proc. Natl. Acad. Sci. USA, *96:* 10869–10874, 1999.

31. Reiter, J. L., Threadgill, D. W., Eley, G. D., Strunk, K. E., Danielsen, A. J., Sinclair, C. S., Pearsall, R. S., Green, P. J., Yee, D., Lampland, A. L., Balasubramaniam, S., Crossley, T. D., Magnuson, T. R., James, C. D., and Maihle, N. J. Comparative genomic sequence analysis and isolation of human and mouse alternative *EGFR* transcripts encoding truncated receptor isoforms. Genomics, *71:* 1–20, 2001.

Downloaded from http://aacrjournals.org/cebp/article-pdf/12/2/103/1743151/ce0203000103.pdf by guest on 05 November 2024

32. Baron, A. T., Lafky, J. M., Connolly, D. C., Peoples, J., O'Kane, D. J., Suman, V. J., Boardman, C. H., Podratz, K. C., and Maihle, N. J. A sandwich type acridinium-linked immunosorbent assay (ALISA) detects soluble ErbB1 (sErbB1) in normal human sera. J. Immunol. Methods, *219:* 23–43, 1998.

33. Baron, A. T., Lafky, J. M., Boardman, C. H., Balasubramaniam, S., Suman, V. J., Podratz, K. C., and Maihle, N. J. Serum sErbB1 and epidermal growth factor levels as tumor biomarkers in women with stage III or IV epithelial ovarian cancer. Cancer Epidemiol. Biomark Prev., *8:* 129–137, 1999.

34. Baron, A. T., Lafky, J. M., Suman, V. J., Hillman, D. W., Buenafe, M. C., Boardman, C. H., Podratz, K. C., Perez, E. A., and Maihle, N. J. A preliminary study of serum concentrations of soluble epidermal growth factor receptor (sErbB1), gonadotropins, and steroid hormones in healthy men and women. Cancer Epidemiol. Biomark Prev., *10:* 1175–1185, 2001.

35. Gill, G. N., and Weber, W. Purification of functionally active epidermal growth factor receptor protein using a competitive antagonist monoclonal antibody and competitive elution with epidermal growth factor. Methods Enzymol., *146:* 82–88, 1987.

36. Baron, A. T., Huntley, B. K., Lafky, J. M., Reiter, J. L., Liebenow, J., McCormick, D. J., Ziesmer, S. C., Roche, P. C., and Maihle, N. J. Monoclonal antibodies specific for peptide epitopes of the epidermal growth factor receptor's extracellular domain. Hybridoma, *16:* 259–271, 1997.

37. Malkasian, G. D., Jr., Podratz, K. C., Stanhope, C. R., Ritts, R. E., Jr., and Zurawski, V. R., Jr. CA 125 in gynecologic practice. Am. J. Obstet. Gynecol., *155:* 515–518, 1986.

38. Niloff, J. M., Knapp, R. C., Lavin, P. T., Malkasian, G. D., Berek, J. S., Mortel, R., Whitney, C., Zurawski, V. R. J., and Bast, R. C. J. The CA 125 assay as a predictor of clinical recurrence in epithelial ovarian cancer. Am. J. Obstet. Gynecol., *155:* 56–60, 1986.

39. Zurawski, V. R., Jr., Orjaseter, H., Andersen, A., and Jellum, E. Elevated serum CA 125 levels prior to diagnosis of ovarian neoplasia: relevance for early detection of ovarian cancer. Int. J. Cancer, *42:* 677–680, 1988.

40. Talbot, R. W., Jacobsen, D. J., Nagorney, D. M., Malkasian, G. D., and Ritts, R. E., Jr. Temporary elevation of CA 125 after abdominal surgical treatment for benign disease and cancer. Surg. Gynecol. Obstet., *168:* 407–412, 1989.

41. Hanley, J. A., and McNeil, B. J. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology, *143:* 29–36, 1982.

42. Ullrich, A., Coussens, L., Hayflick, J. S., Dull, T. J., Gray, A., Tam, A. W., Lee, J., Yarden, Y., Libermann, T. A., Schlessinger, J., Downward, J., Mayes, E. L. V., Whittle, N., Waterfield, M. D., and Seeburg, P. H. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature (Lond.), *309:* 418–425, 1984.

43. Salvatori, L., Ravenna, L., Felli, M. P., Cardillo, M. R., Russo, M. A., Frati, L., Gulino, A., and Petrangeli, E. Identification of an estrogen-mediated deoxyribonucleic acid-binding independent transactivation pathway on the epidermal growth factor receptor gene promoter. Endocrinology, *141:* 2266–2274, 2000.

44. Yarden, R. I., Lauber, A. H., El-Ashry, D., and Chrysogelos, S. A. Bimodal regulation of epidermal growth factor receptor by estrogen in breast cancer cells. Endocrinol., *137:* 2739–2747, 1996.

45. Yarden, R. I., Wilson, M. A., and Chrysogelos, S. A. Estrogen suppression of EGFR expression in breast cancer cells: a possible mechanism to modulate growth. J. Cell. Biochem., *36*(Suppl)*:* 232–246, 2001.

46. Armstrong, D. G., and Webb, R. Ovarian follicular dominance: the role of intraovarian growth factors and novel proteins. Rev. Reprod., *2:* 139–146, 1997.

47. McBean, J. H., Brumsted, J. R., and Stirewalt, W. S. *In vivo* estrogen regulation of epidermal growth factor receptor in human endometrium. J. Clin. Endocrinol. Metab., *82:* 1467–1471, 1997.

48. Granai, C. O., Gajewski, W. H., and Arena, B. Ovarian cancer: issues and management. Cancer J., *7:* 7–11, 1994.

49. Harlap, S. The epidemiology of ovarian cancer. *In:* M. Markman and W. J. Hoskins (eds.), Cancer of the Ovary, pp. 79–93. New York, NY: Raven Press, Ltd., 1993.

50. Meden, H., Marx, D., Fattahi, A., Rath, W., Kron, M., Wuttke, W., Schauer, A., and Kuhn, W. Elevated serum levels of *c-erbB-2* oncogene product in ovarian cancer patients and in pregnancy. J. Cancer Res. Clin. Oncol., *129:* 378–381, 1994.

51. Meden, H., Marx, D., Schauer, A., Wuttke, W., and Kuhn, W. Prognostic significance of p105 (*c-erbB-2*. HER2/*neu*) serum levels in patients with ovarian cancer. Anticancer Res., *17:* 757–760, 1997.

52. Cheung, T. H., Wong, Y. F., Chung, T. K. H., Maimonis, P., and Chang, A. M. Z. Clinical use of serum c-erbB-2 in patients with ovarian masses. Gynecol. Obstet. Investig., *48:* 133–137, 1999.

53. Yazici, H., Dolapcioglu, K., Buyru, F., and Dalay, N. Utility of c-erbB-2 expression in tissue and sera of ovarian cancer patients. Cancer Investig., *18:* 110–114, 2000.

54. Skirnisdottir, I., Sorbe, B., and Seidal, T. The growth factor receptors HER-2/neu and EGFR, their relationship, and their effects on the prognosis in early stage (FIGO I-II) epithelial ovarian carcinoma. Int. J. Gynecol. Cancer, *11:* 119–129, 2001.

55. Cole, P., and Morrison, A. S. Basic issues in population screening for cancer. J. Natl. Cancer Inst., *64:* 1263–1272, 1980.

56. Mori, M., and Miyake, H. Dietary and other risk factors of ovarian cancer among elderly women. Jpn. J. Cancer Res., *79:* 997–1004, 1988.

57. Risch, H. A., Jain, M., Marrett, L. D., and Howe, G. R. Dietary fat intake and risk of epithelial ovarian cancer. J. Natl. Cancer Inst., *86:* 1409–1415, 1994.

58. Glud, E., Kjaer, S. K., Troisi, R., and Brinton, L. A. Fertility drugs and ovarian cancer. Epidemiol. Rev., *20:* 237–257, 1998.

59. Kushi, L. H., Mink, P. J., Folsom, A. R., Anderson, K. E., Zheng, W., Lazovich, D., and Sellers, T. A. Prospective study of diet and ovarian cancer. Am. J. Epidemiol., *149:* 21–31, 1999.

60. Ness, R. B., Grisso, J. A., Cottreau, C., Klapper, J., Vergona, R., Wheeler, J. E., Morgan, M., and Schlesselman, J. J. Factors related to inflammation of the ovarian epithelium and risk of ovarian cancer. Epidemiology, *11:* 111–117, 2000.

61. Helzlsouer, K. J., Bush, T. L., Alberg, A. J., Bass, K. M., Zacur, H., and Comstock, G. W. Prospective study of serum CA-125 levels as markers of ovarian cancer. JAMA, *269:* 1123–1126, 1993.

62. Jacobs, I. J., Skates, S., Davies, A. P., Woolas, R. P., Jeyerajah, A., Weidemann, P., Sibley, K., and Oram, D. H. Risk of diagnosis of ovarian cancer after raised serum ca 125 concentration–a prospective cohort study. Br. Med. J., *313:* 1355–1358, 1996.

63. Menon, U., Talaat, A., Jeyarajah, A. R., Rosenthal, A. N., MacDonald, N. D., Skates, S. J., Sibley, K., Oram, D. H., and Jacobs, I. J. Ultrasound assessment of ovarian cancer risk in postmenopausal women with CA125 elevation. Br. J. Cancer, *80:* 1644–1647, 1999.

64. Hennekens, C. H., and Buring, J. E. Screening. *In:* S. L. Mayrent (ed.), Epidemiology in Medicine, pp. 327–347. Boston, MA: Little, Brown and Company, 1987.

65. Pepe, M. S., Etzioni, R., Feng, Z. D., Potter, J. D., Thompson, M. L., Thornquist, M., Winget, M., and Yasui, Y. Phases of biomarker development for early detection of cancer. J. Natl. Cancer Inst., *93:* 1054–1061, 2001.

66. Xu, Y., Shen, Z., Wiper, D. W., Wu, M., Morton, R. E., Elson, P., Kennedy, A. W., Belinson, J., Markman, M., and Casey, G. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. JAMA, *280:* 719–723, 1998.

67. Udagawa, Y., Aoki, D., Ito, K., Uejima, T., Uemura, M., and Nozawa, S. Clinical characteristics of a newly developed ovarian tumour marker, galactosyltransferase associated with tumour (GAT). Eur. J. Cancer, *34:* 489–495, 1998.

68. van Haaften-Day, C., Shen, Y., Xu, F., Yu, Y., Berchuck, A., Havrilesky, L. J., de Bruijn, H. W., van der Zee, A. G., Bast, R. C., Jr., and Hacker. N. F. OVX1, macrophage-colony stimulating factor, and CA-125-II as tumor markers for epithelial ovarian carcinoma: a critical appraisal. Cancer (Phila.), *92:* 2837–2844, 2001.

69. Mok, S. C., Chao, J., Skates, S., Wong, K-K., Yiu, G. K., Muto, M. G., Berkowitz, R. S., and Cramer, D. W. Prostasin, a potential serum marker for ovarian cancer: identification through microarray technology. J. Natl. Cancer Inst., *93:* 1458–1464, 2001.

70. Kim, H., Scorilas, A., Katsaros, D., Yousef, G. M., Massobrio, M., Fracchioli, S., Piccinno, R., Gordini, G., and Diamandis, E. P. Human kallikrein gene 5 (KLK5) expression is an indicator of poor prognosis in ovarian cancer. Br. J. Cancer, *84:* 643–650, 2001.

71. Diamandis, E. P., Okui, A., Mitsui, S., Luo, L. Y., Soosaipillai, A., Grass, L., Nakamura, T., Howarth, D. J., and Yamaguchi, N. Human kallikrein 11: a new biomarker of prostate and ovarian carcinoma. Cancer Res., *62:* 295–300, 2002.

72. Petricoin, E. F., Ardekani, A. M., Hitt, B. A., Levine, P. J., Fusaro, V. A., Steinberg, S. M., Mills, G. B., Simone, C., Fishman, D. A., Kohn, E. C., and Liotta, L. A. Use of proteomic patterns in serum to identify ovarian cancer. Lancet, *359:* 572–577, 2002.

73. Tuxen, M. K., Soletormos, G., and Dombernowsky, P. Tumor markers in the management of patients with ovarian cancer. Cancer Treat. Rev., *21:* 215–245, 1995.

74. von Schlippe, M., and Rustin, G. J. Circulating tumour markers in ovarian tumours. Forum Trends in Experimental and Clinical Medicine, *10:* 383–392, 2000.

75. Zurawski, V. R., Jr., Broderick, S. F., Pickens, P., Knapp, R. C., and Bast, R. C., Jr. Serum CA 125 levels in a group of nonhospitalized women: relevance for the early detection of ovarian cancer. Obstet. Gynecol., *69:* 606–611, 1987.

76. Einhorn, N., Sjovall, K., Knapp, R. C., Hall, P., Scully, R. E., Bast, R. C., Jr., and Zurawski, V. R., Jr. Prospective evaluation of serum CA 125 levels for early detection of ovarian cancer. Obstet. Gynecol., *80:* 14–18, 1992.

77. Malkasian, G. D., Jr., Knapp, R. C., Lavin, P. T., Zurawski, V. R., Jr. Podratz, K. C., Stanhope, C. R., Mortel, R., Berek, J. S., Bast, R. C., Jr., and Ritts, R. E. Preoperative evaluation of serum CA 125 levels in premenopausal and postmenopausal patients with pelvic masses: discrimination of benign from malignant disease. Am. J. Obstet. Gynecol., *159:* 341–346, 1988.

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