A Preliminary Study of Serum Concentrations of Soluble Epidermal Growth Factor Receptor (sErbB1), Gonadotropins, and Steroid Hormones in Healthy Men and Women

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Abstract

Soluble ErbB (sErbB) growth factor receptors are being investigated as cancer biomarkers. Gonadotropic and steroid hormones have been shown to modulate the expression of ERBB family members in vivo. Accordingly, the range of sErbB1 values and their relationship to gonadotropic and steroid hormones need to be established in healthy subjects to provide a baseline for future clinical studies. We assayed sera from healthy men and women to determine p110 sErbB1 concentrations by acridinium-linked immunosorbent assay (ALISA). Follicle-stimulating hormone (FSH), estradiol, and testosterone concentrations were measured using the ACS:180 Immunoassay Analyzer. Luteinizing hormone (LH) and progesterone concentrations were quantified using the Access® Immunoassay System. Unadjusted for menstrual cycle phase, menopausal status, and exogenous hormone use, p110 sErbB1 concentrations were significantly higher in premenopausal women compared with age-matched men compared with postmenopausal women. Serum sErbB1 concentrations show significant positive associations with FSH concentrations in healthy women and a significant negative association with both FSH and LH concentrations in age-matched men compared with postmenopausal women. Consequently, sErbB1 concentrations are significantly higher in premenopausal women compared with either postmenopausal women or age-matched men and in age-matched men compared with postmenopausal women. Serum sErbB1 concentrations show significant negative associations with both FSH and LH concentrations in healthy women and a significant positive association with FSH concentrations in healthy men. Univariate linear regression models show that these respective gonadotropic and steroid hormones and age are independent predictors of sErbB1 concentrations in men and women. Multivariate models show that when age and FSH and LH concentrations are mutually adjusted for each other, they account for 22% of the variability observed in sErbB1 concentrations in healthy women. These data support the hypothesis that gonadotropic and steroid hormones may modulate ERBB1 expression in vivo and suggest that age- and gonadotropin-adjusted sErbB1 concentrations may be of clinical utility. Furthermore, these data demonstrate that gender, age, menopausal status, and exogenous hormone use must be considered when using serum p110 sErbB1 concentrations as cancer biomarkers.

Introduction

The ERBB family of proto-oncogenes is known to be clinically relevant for a variety of human cancers. This family of genes encodes four structurally related transmembrane receptor tyrosine kinases: the epidermal growth factor receptor (EGFR, ErbB1, and HER1), ErbB2 (HER2 and neu), ErbB3 (HER3), and ErbB4 (HER4) (1, 2). Numerous studies demonstrate that ERBB genes and their receptors are amplified and/or overexpressed in human carcinomas of the breast (3–10), colon and rectum (11–14), endometrium (15), lung (16, 17), ovary (18), and prostate gland (19, 20). Clinically, amplification of ERBB genes and overexpression of ErbB receptors have been associated with disease recurrence and poor patient prognosis (4, 15, 16, 21–31), as well as with decreased responsiveness to hormone therapy and chemotherapy (28, 32–34).

In addition to the full-length transmembrane forms of ErbB receptors, secreted or soluble forms of these receptors (sErbB4) are produced by normal and malignant cells (35). These sErbB proteins contain only the extracellular domain of the receptor. They are produced either by proteolytic cleavage of the full-length receptor (36, 37) or from aberrantly or alternatively spliced mRNA transcripts (38–52). Our laboratory has identified three alternatively spliced mRNA transcripts of the ERBB1 proto-oncogene from human placenta that are 1.8, 2.4, and 3.0 kb in length (49, 52). In vitro, the 1.8- and 3.0-kb transcripts encode mature glycoproteins of about M, 60,000 and M, 110,000, respectively. Furthermore, we have identified a M, 110,000 soluble ErbB1 (p110 sErbB1) protein from human sera (53), and experiments are under way to determine the relation-
ship between this serum protein and the protein encoded by the 3.0-kb ERBB1 transcript (52).

The clinical importance of full-length ErbB receptors has stimulated the investigation of their soluble forms as potential serum biomarkers (54, 55). Immunoassay studies show that serum, urine, or tissue sErbB1 concentrations differ between healthy controls and patients with lung (56–58), gastric (59), and ovarian (60, 61) cancer. Previous studies also have demonstrated that serum or tissue sErbB2 concentrations are elevated in women with breast (62–69) or ovarian (67, 70, 71) cancer, and that elevated serum sErbB2 concentrations correlate with a poor prognosis and shorter overall survival (69, 71). In addition, elevated pretreatment sErbB2 concentrations have been correlated with poor clinical responsiveness to hormone therapy (33, 65, 72) and chemotherapy (73, 74) in metastatic breast cancer patients. Together, these studies suggest that alterations in serum sErbB concentrations may be useful in diagnosing cancer, in monitoring disease recurrence, and in predicting therapeutic responsiveness and disease outcome in cancer patients. It is also possible that serum sErbB concentrations may be useful in assessing exposure to environmental carcinogens and cancer risk or in screening for patients with preclinical disease. Accordingly, the normal range of sErbB1 concentrations needs to be established in healthy subjects to provide a baseline for comparison.

Expression of ERBB proto-oncogenes is modulated by gonadotropic and steroid (75) hormones; this concept is supported by in vitro tissue culture experiments and observations from animal models and humans. For example, estrogen treatment of ZR-75-1 human breast cancer cells has been shown to down-modulate expression of ERBB2 (76), and transfection of MCF-7 breast cancer cells with an inducible plasmid expressing antisense estrogen receptor mRNA resulted in decreased estrogen receptor protein synthesis and a 4-fold increase in ERBB1 gene expression (77). In the rat, ErbB2 receptors in breast tissue increase during lactation, thus suggesting a functional relationship between transcription and translation of the ERBB2 proto-oncogene, breast tissue differentiation, and gonadotropic and steroid hormone regulation (78). In rat ovarian tissue, ErbB1 expression has been shown to increase 2-fold during early estrus, the phase of the ovarian cycle after the pituitary LH/FSH surges, in which estrogen and progesterone synthesis are increased (79). In humans, premenopausal women using hormonal contraception and postmenopausal women using hormone replacement therapy have significantly lower serum p110 sErbB2 concentrations than do premenopausal or postmenopausal women not taking exogenous hormones (80). Finally, estrogen replacement therapy in women with hypoactive ovaries has been shown to result in increased ERBB1 mRNA levels in endometrial tissues (81). Together, these studies demonstrate that gonadotropic and steroid hormone stimulation can yield unique changes in ERBB gene expression patterns in different cell types, tissues, and organs. These peptide and steroid hormones, therefore, have the potential to confound or modify the observed differences in serum sErbB1 concentrations between healthy individuals and patients with cancer. For example, a patient suspected of having cancer or a patient with preclinical disease may need to be stratified first according to menopausal status and/or menstrual cycle phase before using serum sErbB1 concentrations to assess the patient’s cancer status. In this regard, the importance of accounting for menstrual cycle phase when interpreting serum biomarker values has been demonstrated recently for the breast cancer tumor marker, CA 15-3, which displays significant menstrual cycle variability (82). In addition, a patient’s current and/or past use of exogenous hor-

mones, such as oral contraceptives or hormone replacement therapy, may need to be considered when evaluating the significance of changes in serum sErbB1 concentrations. Therefore, an understanding of gonadotropic and steroid hormone influences on serological sErbB1 concentrations is relevant and needed.

Here, we report a preliminary range for serological p110 sErbB1 in both healthy men and women for the ALISA developed by Baron et al. (53). Furthermore, given the evidence that gonadotropic and steroid hormones modulate ERBB gene expression in vivo, we also evaluate whether serum sErbB1 concentrations correlate with gender, age, and gonadotropic and steroid hormone concentrations. The data reported here further support the concept that gonadotropic hormones may modulate ERBB1 expression in vivo. These data also confirm our supposition that gender, age, menopausal status, menstrual cycle phase, and exogenous hormone use need to be considered when evaluating serum p110 sErbB1 as a cancer biomarker.

Materials and Methods

Serum Samples. Blood from 88 healthy men and 143 healthy women was collected 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 (53). Each healthy volunteer was required to provide a recent medical history that included a physical exam and the results of the following tests: hematology group, chemistry group, lipids, thyroid function, and urinalysis. Chest X-ray and electrocardiogram also were performed on age-appropriate subjects. Details from the medical history provided at the time of the blood collection from the healthy men and women are available in the clinical records. These men and women ranged in age from 23 to 79 years (median, 37 years) and 20 to 76 years (median, 47 years), respectively. Briefly, all blood samples were processed into serum and stored at −70°C until they were used. Serum p110 sErbB1 concentrations were quantified for all samples. However, sufficient serum volumes for gonadotropic and steroid hormone concentration measurements were only available for 83 of the 88 healthy men and 127 of the 143 healthy women.

Menopausal Status Determination. The medical records for all of the women in this study were obtained from Medical Information Resources at the Mayo Clinic; this department provides access to detailed archival medical information for all inpatients and outpatients seen at Mayo Clinic since 1909. The following standard criteria were reviewed in the medical records to ascertain menopausal status at the time of the blood draw; patient age, date of last reported menstrual period, self-reported symptoms of menopause (hot flashes, mood swings, and irregular menses), and date of hysterectomy and/or bilateral oophorectomy (83–85). In addition, we used the FSH concentrations measured in this study to assist in determining the patient’s menopausal status. The criteria used to assign menopausal status were two or more of the following: (a) age <60 years; (b) last reported menstrual period <6 months from the date of the blood draw; (c) no self-reported symptoms of menopause; (d) no bilateral oophorectomy; and (e) FSH concentration <30 IU/l (reference interval for ACS:180 Immunoassay Analyzer). The criteria used to assign postmenopausal status were one of the following: (a) age ≥60 years; or (b) bilateral oophorectomy; or two or more of the following: (a) last reported menstrual period >6 months from the date of the blood draw; (b) self-reported symptoms of menopause; and (c)
FSH concentration >36 IU/l (reference interval for ACS:180 Immunoassay Analyzer). Perimenopausal status was assigned to women exhibiting both premenopausal and postmenopausal characteristics (i.e., last reported menstrual period <6 months from the date of the blood draw and self-reported hot flashes and irregular menses). One woman underwent a hysterectomy prior to her blood draw; however, we were not able to determine her ovarian function from the information abstracted from her medical record or from her FSH concentration. Therefore, we were unable to classify this woman’s menopausal status at the time of her blood draw. For the 143 women enrolled in this study, 81 were classified as premenopausal, 2 as perimenopausal, 59 as postmenopausal, and 1 as indeterminate.

\textbf{p110 sErbB1 ALISA.} Serum p110 sErbB1 concentrations were determined by ALISA as outlined by Baron \textit{et al.} (53, 61). This ALISA is characterized by high-sensitivity (intrassay lower limit of detection <1 fmol/ml), a broad linear range (∼1–4,000 fmol/ml), and good reproducibility (coefficients of variance <10%). Also, this ALISA specifically quantifies p110 sErbB1 and does not detect the M, 60,000 protein product of the 1.8-kb transcript (p60 sErbB1), p105 sErbB2, or full-length ErbB2, ErbB3, or ErbB4. Initially, all 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 assay’s standard curve were re-assayed either undiluted or diluted 1:5 in ALBB, whereas serum samples yielding RLUs above the linear range of the assay’s standard curve were re-assayed either diluted 1:20 or 1:50 in ALBB. For each trial, the mean RLUs for each duplicate was determined and a corresponding p110 sErbB1 concentration in fmol/ml was calculated. The reported p110 sErbB1 concentration for each serum sample is the median value from the three trials. The interassay biological detection limit (4.5 SDs above the zero calibrator) for the ALISAs performed in this study was 41 fmol/ml p110 sErbB1. The ALISAs were partially automated using a BIOMEK 1000 laboratory work station (Beckman-Coulter, Fullerton, CA), and acridinium decomposition was measured with a microplate luminometer (model LB 96P; EG&G Berthold Analytical Instruments, Nashua, NH).

\textbf{Gonadotropic and Steroid Immunoassays.} The Mayo Foundation Immunochemical Core Laboratory performed the FSH, LH, estradiol, progesterone, and testosterone immunoassays. Serum FSH, estradiol, and testosterone concentrations were measured according to the manufacturer’s instructions using the ACS:180 Immunoassay Analyzer (Bayer Corporation-Diagnostics Division, Tarrytown, NY), which uses paramagnetic particles as the solid phase and acridinium-based photochemistry. The FSH ACS:180 assay is a chemiluminescent sandwich immunoassay, and the Estradiol-6 and Testosterone ACS:180 assays are competitive chemiluminescent immunoassays. The minimum detectable concentrations of FSH, estradiol, and testosterone according to the ACS:180 Immunoassay Analyzer manufacturer’s instructions are reported to be 0.2 IU/l, 35.0 pg/ml, and 30 ng/dl, respectively.

Serum LH and progesterone concentrations were assayed according to the manufacturer’s specifications using the Access Immunoassay System (Beckman-Coulter, Fullerton, CA). The Access Immunoassay System is an immunoenzymatic assay that also uses paramagnetic particles as the solid phase, but in contrast to the ACS assays, alkaline phosphatase is used to produce the luminescence signal rather than an acridinium-labeled molecule. The Access human luteinizing hormone assay is an immunoenzymatic sandwich assay, and the Access progesterone assay is a competitive immunoenzymatic assay.

The minimum detectable concentrations of human luteinizing hormone and progesterone according to the manufacturer’s specifications are reported to be 0.2 IU/l and 0.08 ng/ml, respectively.

\textbf{Statistical Analyses.} The Wilcoxon rank-sum test was used to determine whether significant differences exist between p110 sErbB1 concentrations in men and women, as well as between p110 sErbB1 concentrations in premenopausal and postmenopausal women. The Wilcoxon signed-rank test was used to determine whether significant differences exist between p110 sErbB1 concentrations in premenopausal and postmenopausal women and age-matched men. Spearman’s rank-order correlation coefficient (rho) and linear regression analyses were used to determine whether associations between p110 sErbB1 concentrations and age or gonadotropic hormone and steroid concentrations exist.

\textbf{Results} \textbf{Serum p110 sErbB1 Concentrations Differ According to Gender, Age, and Menopausal Status.} When unadjusted for age, serum p110 sErbB1 concentrations do not differ significantly between healthy men and women (Fig. 1 and Table 1). However, we observed significant positive (rho = 0.49) and negative (rho = −0.34) associations between serum sErbB1 concentrations and age in healthy men and women, respectively (Fig. 2). Visual comparison of sErbB1 concentrations versus age revealed that sErbB1 concentrations tend to increase as men age (Fig. 2A), whereas sErbB1 concentrations tend to decrease as women age (Fig. 2B). Further analysis of sErbB1 concentrations with regard to menopausal status showed that sErbB1 concentrations are significantly higher in premenopausal women than in postmenopausal women (Fig. 3A and Table 1). To determine whether this difference is simply related to age, we next age-matched 1 male to 1 female so that each pair was within 3 years of age at the time of their blood draw, and so that their blood draws were within 2 years of each other. Interestingly, serum sErbB1 concentrations are significantly higher in premenopausal women than in age-matched men, and they are significantly lower in postmenopausal women than in age-matched men (Fig. 3B and Table 1). These observations indicate that age-dependent differences in serum sErbB1 concentrations exist between men and women. However, these data
Serum Levels of sErbB1, Gonadotropins, and Steroid Hormones

Marked as shown.

Interassay biological detection limit for the ALISAs performed in this study is the median value for one serum sample assayed three times in duplicate. The women (n) increase significantly with age, whereas serum sErbB1 concentrations of healthy men (n) are plotted against age. Serum sErbB1 concentrations of healthy men (n) ranged from 0.16 to 0.13. However, when these data are stratified on the basis of gender, we observed a weak positive association between p110 sErbB1 and FSH concentrations in healthy men (rho = 0.22), and little or no association between sErbB1 and LH (rho = 0.10), estradiol (rho = 0.05), progesterone (rho = 0.07), or testosterone (rho = −0.02) concentrations (Fig. 4). In contrast, these data show weak negative associations between sErbB1 concentrations versus both FSH (rho = −0.29) and LH (rho = −0.19) concentrations in healthy women (Fig. 4). Little or no association between sErbB1 concentrations versus estradiol (rho = 0.16), progesterone (rho = 0.13), or testosterone (rho = 0.02) concentrations are seen in healthy women (Fig. 4). Finally, when women are stratified by menopausal status, these data show no significant association between sErbB1 concentrations and FSH, LH, estradiol, progesterone, or testosterone concentrations in either healthy premenopausal (rho ranged from −0.01 to 0.15) or postmenopausal women (rho ranged from −0.06 to 0.22). Taken together, these data support the concept that circulating gonadotropic and steroid hormones, and in particular FSH and LH, may modulate sErbB1 expression in vivo.

To better understand the gender-specific associations between p110 sErbB1 concentrations versus FSH and LH concentrations, and sErbB1 concentrations versus age, we performed linear regression analyses of these data in men and women. Univariate linear regression shows that age (R² = 0.221135; P < 0.0001) and FSH concentrations (R² = 0.055203; P = 0.0325) are significant but poor independent predictors of sErbB1 concentrations in men, accounting for only 22% and 5% of the variability in sErbB1 concentrations, respectively. Multivariate linear regression with age (P = 0.0003) and FSH concentrations (P = 0.9781) included in the model shows that FSH concentrations are not significant predictors of sErbB1 concentrations when adjusted for age in men (R² = 0.198468). In women, univariate linear regression shows that age (R² = 0.146610; P < 0.0001), FSH concentrations (R² = 0.113097; P < 0.0001), and LH concentrations (R² = 0.040638; P = 0.023) are significant but poor independent predictors of sErbB1 concentrations, accounting for only 14%, 11%, and 4% of the variability in sErbB1 concentrations, respectively. Multivariate linear regression with age (P = 0.0043), FSH concentrations (P = 0.0579; borderline significance), and LH concentrations (P = 0.0241) included in the model shows that when all three covariates are mutually adjusted for each other, they account for 22% of the variability observed in sErbB1 concentrations in healthy women (R² = 0.222938). Circulating FSH and LH concentrations increase slightly with age in men and dramatically in women in conjunction with menopause (83–87). Because sErbB1 concentrations also change with age in men and women, we compared

![Fig. 2. Serum p110 sErbB1 concentrations in healthy men (A) and women (B) are plotted against age. Serum sErbB1 concentrations of healthy men (n = 88; A) increase significantly with age, whereas serum sErbB1 concentrations of healthy women (n = 143; B) decrease significantly with age. Each data point represents the median value for one serum sample assayed three times in duplicate. The interassay biological detection limit for the ALISAs performed in this study is marked as shown.](https://cebp.aacrjournals.org)
serum concentrations of sErbB1 between healthy premenopausal women (n = 63) and age-matched men (n = 22) differ significantly (P = 0.0271). Horizontal lines, median serum sErbB1 concentration for these groups of postmenopausal women (3,181 fmol/ml) and age-matched men (5,661 fmol/ml).

**Discussion**

Several isoforms of the epidermal growth factor receptor, ranging in molecular weight from M, 80,000 to M, 110,000, have been reported in human tissues and body fluids (43, 44, 49, 52, 53, 58, 61). Ilekis et al. (44) have used immuno-affinity and EGF-affinity chromatography to purify a truncated M, 80,000 ErbB1 protein from human term placenta, and Witters et al. (58) have identified a M, 95,000 sErbB1 isoform in human urine by immunoprecipitation and Western blot analysis. The mechanisms for generating these p80 and the p95 ErbB1 human isoforms, whether by proteolytic cleavage or by alternative mRNA transcription, have not yet been elucidated. Also, it currently is not known whether these proteins are present in human circulatory fluids. Reiter and co-workers have isolated and sequenced two alternative epidermal growth factor receptor mRNA transcripts of 1.8 kb (49) and 3.0 kb (52), which encode P80 and the P95 ErbB1 human isoforms, whether by proteolytic cleavage or by alternative mechanisms for generating these P80 and the P95 ErbB1 human isoforms. We previously have demonstrated that human serum contains a M, 110,000 sErbB1 isoform by immunoprecipitation and Western blot analysis, and that our ALISA specifically measures this p110 sErbB1 isoform (53, 61). Experiments are under way to determine the relationship between the p110 sErbB1 serum protein and the protein encoded by the 3.0-kb ERBB1 transcript (52). In summary, current data indicate that human circulatory fluids contain at least one sErbB1 isoform of M, 110,000.

sErbB1:gonadotropin ratios against age. For healthy men, we observed no association between the sErbB1:FSH ratio versus age (rho = 0.02; data not shown). Strikingly, we observed strong negative associations between both the sErbB1:FSH (rho = −0.77) and sErbB1:LH (rho = −0.73) ratio versus age in healthy women (Fig. 5).

**Different Immunoassays Yield Disparate Serum p110 sErbB1 Concentrations.** Four separate immunoassays have been used to measure sErbB1 (and full-length ErbB1) concentrations in human tissue, body fluids, tissue culture cells, and conditioned culture media. Three of these assays are ELISA kits marketed by Oncogene Research Products, CIS Isotopen-diagnostik GmbH, and Triton Diagnostics, Inc. The fourth assay is the ALISA used in this study (53). Table 2 compares the serum sErbB1 values reported for each immunoassay and statistically significant differences observed between the groups of subjects studied; sErbB1 concentration ranges were reported to include either the entire range of values, values between the 25th to 75th percentiles, or values between the 95% confidence interval. All studies, to date, have reported sErbB1 concentrations in fmol/ml of serum (53, 59, 61, 88–91), with the exception of the study by Streckfus et al. (92), which used the Triton Diagnostics ELISA and reported serum sErbB1 concentrations in fmol/ml of total serum protein. The reported values for serum sErbB1 concentrations ranged from 18 to 1500 fmol/ml (56, 57, 59, 88, 90, 91) and from 27.50 to 38.44 fmol/ml (89) for the Oncogene Science and CIS Isotopen-diagnostik ELISAs, respectively, and from nondetectable to 61,583 fmol/ml for the ALISA (53, 61). Clearly, much higher serum sErbB1 concentrations are revealed by the ALISA than by any of the commercially available assays. Moreover, sErbB1 concentrations measured in the same serum samples with the Oncogene Research Products ELISA do not correlate with those measured by the ALISA (Table 2; Ref. 53). The results of this comparison indicate that the serum sErbB1 concentrations reported for healthy men and women in this study should only be applied to the ALISA developed by Baron et al. (53).
centrations for patients with disease or prior exposure to carcinogens and/or risk factors (56–60, 88, 89, 93), whereas Baron et al. (61) reported significantly lower sErbB1 concentrations for patients with disease (epithelial ovarian cancer). Other studies have found no significant differences in sErbB1 concentrations between cancer patients, exposed workers (uranium miners), and healthy subjects (90–92). Moreover, these published reports have either quantified tissue (60), urine (58), serum (53, 56, 57, 59, 61, 88–92), or plasma (93) sErbB1 concentrations using one of four immunoassays or Western immunoblot methods (60), thereby making it difficult to compare results between studies. Possible explanations for the disparate sErbB1 concentrations reported in these studies including an in-depth comparison of these immunoassays are presented below.

Despite a growing interest in sErbB1 proteins as biomarkers of human cancer, studies to establish a reference range of serum sErbB1 concentrations in healthy men and women have not been performed. Here, we report on the range of values for serum p110 sErbB1 concentrations in healthy men and women as determined by our ALISA (53), and we compare our results with those reported by other investigators using
commercially available sErB1 ELISA kits. We report that our ALISA estimates a broader range of serum sErB1 concentrations than all commercially available ELISA kits, and that the values obtained by our ALISA show no association with those obtained using the Oncogene Research Products ELISA kit on identical serum samples (53). Several methodological and biological mechanisms may explain the broader concentration range of serum sErB1 estimated by our ALISA. In our ALISA, the covalent linkage of the goat antimouse IgG2b-specific polyclonal coupling antibody to the microtiter plate, and/or the use of acridinium-based photochemistry may improve the detectability of captured sErB1 immunocomplex. It also is possible that affinity differences between the capture and detection antibodies for the standard analyte (ErB1) and/or the serum analyte (sErB1), used in each immunoassay, may contribute to the different values reported for sErB1 concentrations from the same serum sample (53). Matrix factors present in human sera, such as serum sErB1 binding proteins or ErB1 ligands (e.g., epidermal growth factor or transforming growth factor-\(\alpha\)) also may differentially affect the performance of these immunoassays. Another possible explanation for the lower concentrations measured by the commercially available enzyme-based immunoassays is that the p110 sErB1 ALISA may detect total sErB1, whereas the ELISAs may detect only free or bound sErB1. Finally, it is possible that the capture and detection antibodies used in each immunoassay detect different serum sErB1 isoforms. These possibilities are particularly intriguing in light of the growing number of immunoassay methods that discriminate between various free, bound, and total PSA isoforms in men with prostate cancer (94–99). Regardless of the mechanism(s) responsible for producing disparate values for serum sErB1 concentrations among these sErB1 immunoassays, it is clear that the range of values reported here for healthy men and women is applicable only to the results obtained with the ALISA developed by Baron et al. (53).

Circulating concentrations of gonadotropic and steroid hormones differ between men and women (86). Moreover, circulating gonadotropic and steroid hormone concentrations in women change with age, pregnancy, lactation, menstrual cycle phase, and menopausal status (87). Although gonadotropic and steroid hormones are thought to regulate expression of the ERBB family in vivo (75, 78–81), no previous studies have examined the relationship between these hormones or their covariates (gender, age, pregnancy, lactation, menstrual cycle phase, and menopausal status) and serum sErB1 concentrations.

Here, we report on serum p110 sErB1 concentrations relative to gender, age, and menopausal status. Unadjusted for age, we observed no statistically significant difference in sErB1 concentrations between healthy men and women ranging in age from 20 to 79 years. However, we observed a strong age-gender interaction with respect to p110 sErB1 concentrations, i.e., serum sErB1 concentrations increase as men but decrease as women age. Univariate linear regression shows that age is a significant but poor independent predictor of sErB1 concentrations in men and women, accounting for only 22% and 14% of the variability in sErB1 concentrations, respectively. Further analyses demonstrate significantly higher serum sErB1 concentrations in premenopausal versus postmenopausal women (53). Our observations, therefore, indicate that age-specific differences in serum sErB1 concentrations exist in men and women. Analogously, age-specific PSA reference ranges have been shown to be important in prostate cancer screening, where age-specific ranges make the PSA test more sensitive in younger men and more specific in older men (100, 101). Our observations, therefore, suggest that prospective studies are needed to define better age-specific sErB1 reference ranges in both healthy men and women.

Our comparison of the differences between sErB1 concentrations in healthy premenopausal versus postmenopausal women or healthy premenopausal and postmenopausal women versus age-matched men, lend support to the concept that serum sErB1 concentrations may be regulated by circulating gonadotropic and steroid hormones. Here, we report on serum p110 sErB1 concentrations relative to FSH, LH, estradiol, progesterone, and testosterone concentrations in healthy men and women. Unfortunately, the ACS:180 immunoassays for estradiol and testosterone yielded many nondetectable values for these hormones in men and postmenopausal women because of lack of assay sensitivity. These nondetectable values for estradiol and testosterone concentrations may have limited our ability to discern any weak associations between sErB1 concentrations and these hormones in either men or women; therefore, we cannot rule out the possibility that associations exist between sErB1 concentrations and estradiol or testosterone. When these data are stratified according to gender, a weak positive association between sErB1 and FSH concentrations is revealed in healthy men. Univariate linear regression shows that FSH concentrations are significant but poor independent predictors of sErB1 concentrations in men, accounting for just 5% of the variability in sErB1 concentrations. However, multivariate linear regression shows that FSH concentrations are not significant predictors of sErB1 concentrations when adjusted for age in men. In contrast, we observed weak negative

Fig. 5. Serum sErB1:FSH (A) and sErB1:LH (B) ratios for healthy women \((n = 127)\) are graphed against age. Strong negative associations between both the sErB1:FSH and sErB1:LH ratio versus age are seen. Spearman’s rank-order correlation coefficient (rho) is given in each graph (r). Premenopausal (○), perimenopausal and indeterminate (△), and postmenopausal (×) women are shown by different symbols.
associations between sErbB1 concentrations and both FSH and LH concentrations in healthy women. In women, univariate linear regression shows that FSH and LH concentrations are significant but poor independent predictors of sErbB1 concentrations, accounting for only 11% and 4% of the variability in sErbB1 concentrations, respectively. However, multivariate linear regression shows that when age, and FSH and LH concentrations are mutually adjusted for each other, they account for 22% of the variability observed in sErbB1 concentrations in healthy women. Together, these data further support the concept that the milieu of circulating gonadotropin and steroid hormones may modulate gender-dependent and age-specific differences in sErbB1 concentrations.

We surmise that the association between sErbB1 and gonadotropin hormone concentrations may be of potential clinical interest. We have observed that serum sErbB1 levels are significantly lower in patients with stage III or IV epithelial ovarian cancer than in healthy women (61). Moreover, ovarian neoplasms are known to be associated with high levels of circulating gonadotropins (102). We hypothesize that age- and gonadotropin-adjusted sErbB1 concentrations or age-specific sErbB1/gonadotropin ratios may differ between healthy women and cancer patients, and that these adjusted values or ratios may be useful in the diagnostic evaluation of patients with epithelial ovarian cancer, in particular. Hence, we propose the need for prospective studies to define better the age- and gonadotropin-adjusted sErbB1 reference ranges in healthy women.

Taken together, the data reported in this study suggest that circulating gonadotropin and steroid hormones may modulate serum p110 sErbB1 concentrations. We propose that further

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<th>Table 2</th>
<th>Comparison of previously reported serum sErbB1 concentrations</th>
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<td>Mean (± SD) sErbB1 (fmol/ml)</td>
<td>Median sErbB1 (fmol/ml)</td>
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<td>Oncogene Research Products ELISA</td>
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<td>Partanen et al. (56, 57), 1994</td>
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<tr>
<td>Unexposed surgical patients (n = 20)</td>
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<td>Healthy asbestos exposed workers (n = 72)</td>
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<td>Asbestos workers with cancer (n = 38)</td>
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<td>Choi et al. (59), 1997</td>
<td></td>
</tr>
<tr>
<td>Healthy men and women (n = 29)</td>
<td>440 (± 46)</td>
</tr>
<tr>
<td>Gastric cancer (n = 26 men, n = 14 women)</td>
<td>681 (± 226)</td>
</tr>
<tr>
<td>Baron et al. (53), 1998</td>
<td></td>
</tr>
<tr>
<td>Healthy men (n = 20)</td>
<td>104</td>
</tr>
<tr>
<td>Healthy women (n = 20)</td>
<td>109</td>
</tr>
<tr>
<td>Lahat et al. (88), 1999</td>
<td></td>
</tr>
<tr>
<td>Unexposed healthy relatives (n = 70)</td>
<td>409</td>
</tr>
<tr>
<td>Exposed asbestos workers (n = 300)</td>
<td>800</td>
</tr>
<tr>
<td>Schneider et al. (90, 91), 1999</td>
<td></td>
</tr>
<tr>
<td>Healthy subjects (n = 23)</td>
<td>34</td>
</tr>
<tr>
<td>Uranium miners without cancer (n = 106)</td>
<td>37</td>
</tr>
<tr>
<td>Uranium miners with lung cancer (n = 21)</td>
<td>37</td>
</tr>
<tr>
<td>Non-miners with lung cancer (n = 88)</td>
<td>37</td>
</tr>
<tr>
<td>Nonmalignant lung disease (n = 50)</td>
<td>41</td>
</tr>
<tr>
<td>CIS Isotopendiagnostik GmbH ELISA</td>
<td></td>
</tr>
<tr>
<td>Birk et al. (89), 1999</td>
<td></td>
</tr>
<tr>
<td>Surgery patients (n = 71)</td>
<td>35.1 (± 5.4)</td>
</tr>
<tr>
<td>Chronic pancreatitis (n = 35)</td>
<td>28.0 (± 10.4)</td>
</tr>
<tr>
<td>Pancreatic cancer (n = 31)</td>
<td>36.7 (± 5.4)</td>
</tr>
<tr>
<td>ALISA</td>
<td></td>
</tr>
<tr>
<td>Baron et al. (53), 1998</td>
<td></td>
</tr>
<tr>
<td>Healthy men (n = 40)</td>
<td>26,411</td>
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<tr>
<td>Healthy women (n = 40)</td>
<td>9,814</td>
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<tr>
<td>Healthy men (n = 20)</td>
<td>26,077</td>
</tr>
<tr>
<td>Healthy women (n = 20)</td>
<td>11,307</td>
</tr>
<tr>
<td>Baron et al. (61), 1999</td>
<td></td>
</tr>
<tr>
<td>Healthy women (n = 21)</td>
<td>8,166</td>
</tr>
<tr>
<td>Ovarian cancer, preoperative (n = 21)</td>
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<tr>
<td>Healthy women (n = 73)</td>
<td>8,298</td>
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<tr>
<td>Ovarian cancer, postoperative (n = 73)</td>
<td>2,695</td>
</tr>
<tr>
<td>Triton Diagnostics ELISA</td>
<td></td>
</tr>
<tr>
<td>Streckfus et al. (92), 2000</td>
<td></td>
</tr>
<tr>
<td>Benign breast conditions (n = 8)</td>
<td>3.52 (± 1.89)</td>
</tr>
<tr>
<td>Breast cancer (n = 12)</td>
<td>5.63 (± 3.10)</td>
</tr>
</tbody>
</table>

*The range of serum sErbB1 concentration was reported to include either the minimum and maximum observed values, values between the 25%–75% percentiles (λ), or values between the 95% confidence interval (*). ND, nondetectable; NS, not significant.

b Student’s t test.
c These serum samples were quantified using both the Oncogene ELISA and the ALISA developed by Baron et al. (53) in 1998. These statistics were not reported in 1998 but are included here for completeness.
d Wilcoxon rank-sum test.
prospective studies are needed to assess whether associations exist between sErbB1 and testosterone or estrogen concentrations and to determine precise reference intervals for sErbB1 concentrations and sErbB1:gonadotropin ratios. Moreover, these reference intervals should account for gender, age, menopausal status, menstrual cycle phase, and exogenous hormone therapy. Concerning exogenous hormone use, we are currently abstracting the medical records of the women in this study for information about prior and current oral contraceptive pill use or hormone replacement therapy at the time of their blood draw to assess whether associations exist between sErbB1 concentrations and these hormonal exposures. In addition, we propose that a prospective cross-sectional study that incorporates multiple blood draws from the same individuals over time is needed to determine the extent of intra- and interindividual variability on sErbB1 concentrations. This study would be useful for evaluating whether fixed genetic traits (e.g., gene mutations) or dynamic short (e.g., blood pressure changes, ingestion of food, and circadian cycles), intermediate (menstrual cycle), and long-term (e.g., aging, menopause, and somatic mutations caused by gene-environment interactions) biological processes may affect serum sErbB1 variability. In conclusion, these data indicate that well-designed epidemiological and clinical studies are necessary to ascertain further the utility of serum p110 sErbB1 in assessing cancer risk, diagnosing and monitoring cancer progression, and/or predicting therapeutic response and disease outcome for cancer.

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References
Serum Levels of sErbB1, Gonadotropins, and Steroid Hormones


A Preliminary Study of Serum Concentrations of Soluble Epidermal Growth Factor Receptor (sErbB1), Gonadotropins, and Steroid Hormones in Healthy Men and Women


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