ONCOLOGY

Validation of serum biomarkers for detection of early- and late-stage endometrial cancer

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OBJECTIVE: The objective of this study was to determine the efficacy of 3 previously described ovarian cancer serum biomarkers (apolipoprotein-1 [ApoA-I], prealbumin [TTR], transferrin [TF]) in the detection of endometrioid and papillary serous adenocarcinoma of the endometrium.

STUDY DESIGN: ApoA-I, TTR, and TF levels were measured in serum samples that were obtained from 433 individuals that included 90 women with normal endometrium, 210 women with early-stage endometrial cancer, and 133 women with late-stage endometrial cancer. Multivariate regression models were constructed to evaluate the usefulness of the biomarkers in the detection of endometrial cancer.

RESULTS: ApoA-I, TTR, and TF distinguished normal samples from early-stage endometrial cancer with a sensitivity of 71% (specificity, 88%) and normal samples from late stage endometrial cancer with a sensitivity of 82% (specificity, 86%).

CONCLUSION: The biomarker panel that consists of ApoA-I, TTR, and TF may prove to be a useful clinical tool for the detection of endometrial cancer.

Key words: biomarker, early stage, endometrial cancer, late stage

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Endometrial carcinoma is the most common gynecologic cancer in the United States, with >7400 deaths per year[1] and a death rate that has doubled since 1985.[2] The increase in mortality rates in women with endometrial cancer is related to an increase in patients with advanced-stage cancers and a larger proportion of high-risk histologic conditions.[3] Current diagnostic approaches rely on endometrial sampling of macroscopic disease that is driven by the presence of symptoms and signs that include vaginal bleeding and metrorrhagia. The key to an improved progression-free survival and overall survival[3] is the early diagnosis of microinvasive disease, which is curable more readily with the use of either hormonal or surgical treatment modalities.[4]

To date, there are no reliable serum biomarkers in clinical use for the detection of endometrioid, clear cell, or papillary serous adenocarcinoma of the endometrium. Serum cancer antigen 125 (CA125) is a high molecular weight glycoprotein and serum CA125 levels ≤35 units/mL are accepted as normal.[5,6] CA125 is currently only relatively accurate in the detection of late stage ovarian cancer (papillary serous histologic subtype), is not used for the detection of endometrial cancer.[7-11]

Although novel proteomic-based approaches have been used successfully to detect and diagnose a number of different types of cancers,[12] none have been reported for endometrial cancer.[13] We previously analyzed protein profiles using surface enhanced laser desorption and ionization time-of-flight mass spectroscopy and identified 3 serum proteins (namely, apolipoprotein-1 [ApoA-I], prealbumin [TTR], and transferrin [TF]) for the early detection of ovarian cancer.[14-16] Because endometrioid adenocarcinoma of the ovary is histologically similar to endometrioid adenocarcinoma of the endometrium, we sought to examine whether the 3 markers that were previously described for ovarian cancer may prove to be useful in the diagnosis of early- or late-stage endometrial cancer.

Materials and Methods

Serum samples were obtained through the Gynecological Oncology Group (GOG) and Cooperative Human Tissue Network. Samples were collected preoperatively according to the standard GOG protocol (GOG-199 protocol). The levels of each individual protein marker (CA125, ApoA-I, TTR, TF) were measured on all serum samples, as previously described.[14] The immunoassay analyzer (Immulite 1000; Siemens Healthcare Diagnostics, Deerfield, IL) was used to measure CA125 level with chemiluminescence technol-

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TABLE 1
Clinical characteristics and age distribution of 433 study samples

		Age			Pathologic condition		
Diagnostic group	n	Mean	SD	Median	Endometrioid adenocarcinoma	Clear cell	Serous carcinoma
Normal	90	44.6	11.7	45			
Early stage	210	63.7	12.1	63	162	10	38
Late stage	133	66.7	13.0	67	55	13	65

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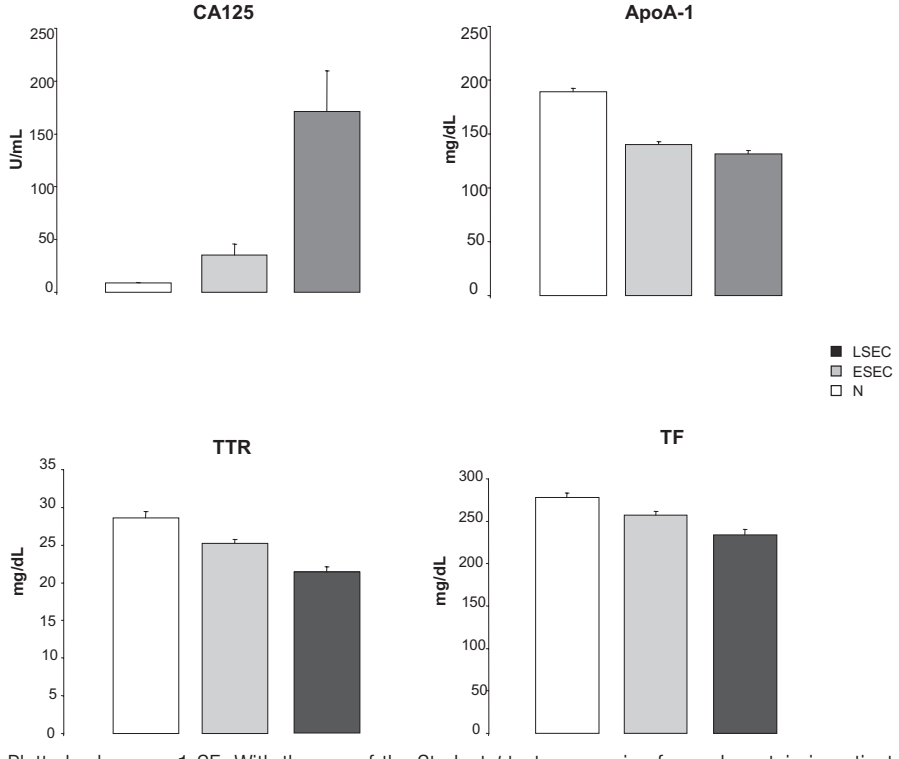
ogy, and the chemistry analyzer (Hitachi 912; Roche Diagnostics, Basel, Switzerland) was used to measure ApoA-I, TTR, and TF levels based on immunoturbimetry technology, as previously described.[14] The reagents were purchased from Diagnostics Product Corporation and Roche.

The 433 serum samples that were used in this study included samples from 90 women with normal uteri (N), 210 women with early-stage endometrial cancer (ESEC), and 133 women with late-stage endometrial cancer (LSEC). *ESEC* was defined as stages I-II. *LSEC* was defined as stages III-IV. All patients were appropriately staged according to the GOG Surgical Manual. The age and pathologic distribution of the samples are provided in Table 1.

FIGURE
Serum levels of 4 markers (cancer antigen 125 [*CA125*], apolipoprotein-1 [*ApoA-I*], prealbumin [*TTR*], transferrin [*TF*])



Plotted values are 1 SE. With the use of the Student *t* test, expression for each protein in patient samples significantly differed from normal samples; all probability values were $< .01$.

ESEC, early-stage endometrial cancer; *LSEC*, late-stage endometrial cancer; *N*, normal.

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Each cancer stage (eg, ESEC and LSEC) and its corresponding histologic subcategory (eg, endometrioid, clear cell, and serous adenocarcinoma; together or individually) were compared with the normal group to identify an association to the set of markers of interest (ApoA-I, TTR, TF, and CA125), with the use of multiple logistic regression (MLRM). Based on the MLRM, we computed predicted probabilities of patients with malignant disease. We report sensitivity and specificity for each diagnostic model by identifying the cutoff point of the predicted probability that yields the maximum sum of sensitivity and sensitivity. To validate our models, we used 10-fold cross validation with the largest difference in the area under the curve (AUC) being within 3% of the original model.

Descriptive statistics of the levels of each individual marker (ApoA-I, TTR, TF, and CA-125) were presented as means (SD) to compare marker levels across all sample categories. MLRMs were built to predict normal vs ESEC and normal vs LSEC. All tests were 2-sided and a probability value of $< .05$ was considered statistically significant. All tests were performed with the SAS statistical package (version 9.1; SAS Institute Inc, Cary, NC).

RESULTS

The serum levels of each of the 4 markers (CA125, ApoA-I, TTR, and TF) are significantly different in both patient samples for ESEC and LSEC, when compared with normal serum samples (Figure).

Serum CA125 levels alone correctly detected only 24% of all the endometrial