Comparison of Serum Human Epididymis Protein 4 with Cancer Antigen 125 as a Tumor Marker in Patients with Malignant and Nonmalignant Diseases

Jose M. Escudero,1 Jose M. Auge,1 Xavier Filella,1 Aureli Torne,2 Jaume Pahisa,2 and Rafael Molina1*

BACKGROUND: Human epididymis protein 4 (HE4), a precursor of human epididymis protein, has been proposed as a tumor marker for ovarian cancer. We evaluated HE4 in comparison with cancer antigen 125 (CA 125) in healthy individuals and in patients with benign and malignant diseases.

METHODS: CA 125 and HE4 serum concentrations were determined in 101 healthy individuals, 535 patients with benign pathologies (292 with benign gynecologic diseases) and 423 patients with malignant diseases (127 with ovarian cancers). CA 125 and HE4 cutoffs were 35 kU/L and 140 pmol/L, respectively.

RESULTS: HE4 and CA 125 results were abnormal in 1.1% and 9.9% of healthy individuals and in 12.3% and 37% of patients with benign diseases, respectively. Renal failure was the most common cause of increased HE4 in patients with benign disease, who had significantly higher HE4 concentrations ($P = 0.001$) than patients with other benign diseases. HE4 showed a higher specificity than CA 125 in patients with benign gynecologic diseases, with abnormal concentrations in 1.3% and 33.2% of the patients, respectively. HE-4 concentrations were abnormal primarily in gynecologic cancer and lung cancer. By contrast, CA 125 was increased in many different nonovarian malignancies, including nonepithelial tumors. A significantly higher area under the ROC curve was obtained with HE4 than with CA 125 for differentiating benign from malignant diseases (0.755 vs 0.643) and in the differential diagnosis of gynecologic diseases (0.874 vs 0.722).

CONCLUSIONS: HE4 has significantly higher diagnostic specificity than CA 125, and the combination of CA 125 and HE4 improved the detection of ovarian cancer in all stages and histological types.

© 2011 American Association for Clinical Chemistry

Recently human epididymis protein 4 (HE4) has been proposed as another tumor marker for ovarian cancer. HE4 is a precursor of the protein human epididymis protein, encoded by a gene located in chromosome 20q12-13.1 (19–21). HE4 is frequently overex-
pressed in ovarian cancers (21), but some expression has also been found in pulmonary, endometrial, and breast adenocarcinomas, mesotheliomas, and less often, gastrointestinal, renal, and transitional cell carcinomas (20–22). Results of previous studies have suggested that HE4 has diagnostic sensitivity similar to that of CA 125, but an increased diagnostic specificity in patients with gynecologic malignancies compared with those with a benign gynecologic disease (7, 8, 22). However, little is known about HE4 diagnostic specificity in other benign and malignant conditions, and it is important to consider potential false-positive results observed with other tumor markers suggested for use in ovarian cancer, such as CA 125 (2–4, 7–17).

In this study we aimed to evaluate HE4 serum concentrations in healthy individuals and in patients with benign and malignant diseases of various origins and to compare the clinical utility of HE4 with that of the tumor marker of choice in ovarian cancer, CA 125.

Materials and Methods

PATIENT POPULATION

We determined HE4 serum concentrations in 101 healthy people (age 20–91 years, median 52 years, SE 1.7 years) (65 female, 36 male); 535 patients with benign diseases (411 females, 124 males); and 423 patients with various malignancies. Healthy women study participants included 34 premenopausal women (age 20–49 years, median 42 years, SE 1.4 years) and 31 postmenopausal women (age 47–91 years, median 70 years, SE 2 years). The group with benign diseases included 22 patients with renal failure, 81 with hepatic disease (15 hepatitis and 66 liver cirrhosis); 17 with dermatological disease (8 psoriasis, 5 eczema, 3 pemphigus, and 3 other diseases); 17 with cardiovascular disease (8 heart failure, 4 acute myocardial infarction, 4 myocardopathies, and 1 arrhythmia); 57 with lung disease (25 chronic obstructive pulmonary disease, 19 pneumonia, 5 pulmonary hypertension, 3 asthma, 2 bronchiectasis, 2 pulmonary thromboembolism, and 7 other diseases); 28 with gastrointestinal disease (8 ulcerative colitis, 6 Crohn disease, 4 gastric peptic ulcer, 6 pancreatitis, and 4 other diseases); 21 with autoimmune disease (4 systemic lupus erythematosus, 5 scleroderma, and 11 other diseases including dermatomyositis and Horton arteritis); and 292 with gynecologic disease (144 ovarian cysts, 58 myomas, 66 endometriosis, 13 endometrial polyps, and 11 other diseases). Serum creatinine concentrations that were not within the reference interval [\(>1.3 \text{ mg/dL (}>115 \text{ mmol/L})\] were found in 17 patients with benign diseases other than renal failure: 6 with lung disease, 4 with liver disease, 2 with cardiovascular disease, 2 with autoimmune disease, and 3 with gynecologic disease.

The group with malignant diseases included 147 patients with nongynecologic cancers or lung cancers, all with metastases (or stage IV) according to the TNM (tumor, node, metastasis) classification: 18 gastric cancer, 10 pancreatic cancer, 28 colorectal cancer, 14 hepatocellular cancer, 19 breast cancer, 7 prostatic cancer, 7 urological cancer, 30 hematologic cancers, 9 malignant melanoma, and 5 mesenchymal tumors (2 sarcomas, 3 mesotheliomas). We also studied 49 patients with non–small cell lung cancer (NSCLC), and 28 patients with small cell lung cancer (SCLC), including 7 patients with intrathoracic tumors and 21 patients with extrathoracic tumors. NSCLC patients included 12 patients in stages I–III (9 adenocarcinomas, 2 squamous, and 1 NSCLC) and 37 patients in stage IV (10 squamous, 23 adenocarcinomas, and 4 NSCLC). We also studied 199 patients with gynecologic tumors, classified according to the International Federation of Gynaecology and Obstetrics (23), including 127 ovarian cancers (25 stages I–II, 53 stage III, and 49 stage IV) (31 premenopausal, 96 postmenopausal), 35 endometrial cancers (14 stages I–II, 21 stages III–IV), 19 endocervical cancers (9 stages I–II, 10 stages III–IV) and 18 squamous cell carcinomas of the cervix (4 stages I–II, 14 stages III–IV). Abnormal serum creatinine concentrations were found in 22 patients with malignancies, including 6 with gynecologic cancer (2 ovarian, 3 endometrial, 1 endocervical), 4 with gastrointestinal cancer, 10 with lung cancer (8 NSCLC, 2 SCLC), 1 with breast cancer and 1 with mesothelioma.

LABORATORY METHODS

Blood samples for CA 125 and HE4 analysis were obtained by venous puncture in our hospital, centrifuged, and stored at –70 °C until assayed. Samples from the majority of patients (including those with benign diseases, gynecologic diseases, or cancer) were obtained at diagnosis when they were admitted to the hospital, before any treatment. Other samples, from patients with advanced malignancies, were obtained at diagnosis of metastases, before any treatment for the recurrence. Samples from healthy individuals were obtained from blood donors, or from patients seen annually in the gynecology department. This protocol was approved by the ethics committee of the hospital clinic. Tumor markers were determined by use of a chemiluminescent enzyme immunoassay on an Architect® (Abbott Laboratories), for which the interassay imprecision values for CA 125 were 2.69% (37.9 kU/L), 2.6% (292.9 kU/L), and 1.26% (612 kU/L). Interassay precision values for HE4 were 3.6% (49.3 pmol/L), 4.6% (171.8 pmol/L), and 4.5% (662.8 pmol/L). The Architect cutoffs for CA 125 and HE4 were 35 kU/L and 140 pmol/L, respectively. Serum creatinine concentrations were measured in all participants by use of an alkaline pi-
Results

Results of CA 125 and HE4 measurements in the different populations studied are shown in Table 1. Significantly higher CA 125 serum concentrations were found in women (median 15, SE 1.5 kU/L) than in men (10, 1.9 kU/L) \( (P = 0.01) \) and, according to menopausal status, with significantly higher concentrations \( (P = 0.005) \) in premenopausal women. For HE4, higher concentrations were found in women [54 (4.1) pmol/L] than in men [49.2 (4.8) pmol/L]; higher concentrations were also seen in postmenopausal women, but the difference did not achieve statistical significance. It is interesting to note that significantly \( (P < 0.02) \) lower HE4 serum concentrations were found in women younger than 40 years (median 37.3, SE 6 pmol/L) than in those older than 40 years (median 65.4, SE 5.6 pmol/L).

In patients with benign diseases, serum concentrations of HE4 and CA 125 that were not within reference intervals were found in 12.3% (66 of 535) and 37% (198 of 535) patients, respectively. For HE4, renal failure was the most common cause of increased results, with 33 of the 65 false increases being the result of renal failure \( [17 \text{ of these in patients with other diseases and creatinine concentrations } >1.3 \text{ mg/dL } (>115 \mu \text{mol/L})] \). Patients with renal failure also showed significantly higher HE4 concentrations \( (P = 0.001) \) than patients with other benign diseases, with a wide range of results. Twenty-one of the 65 false increases were from patients with effusions in the absence of renal disease. The other 11 false increases were from patients with liver disease, lung disease, gynecologic disease, or other benign diseases.

For CA 125, the most common cause of increased results was gynecologic disease, with 96 of the 198 false increases falling into this category. Excluding patients with renal failure or those with creatinine concentrations \( >1.3 \text{ mg/dL } (>115 \mu \text{mol/L}) \) (17 patients: 5 liver cirrhosis and 5 lung, 3 gynecologic, 2 cardiovascular, 1 gastrointestinal, 1 dermatologic disease), the presence of effusions \( (P = 0.001) \), liver diseases excluding effusions \( (P = 0.001) \), and lung diseases \( (P = 0.023) \) comprised other sources of false-positive results for both tumor markers (Table 1). It is interesting to note that HE4 diagnostic specificity in patients with benign gynecologic diseases was higher than that for CA 125. In patients with benign gynecologic conditions, concentrations were above the upper limit of the reference interval in 1.3% of patients for HE4 compared to 33.2% for CA 125.

Table 2 shows the HE4 and CA 125 serum concentrations in patients with cancer. Twenty-three patients had renal failure or creatinine serum concentrations \( >1.3 \text{ mg/dL } (>115 \mu \text{mol/L}) \) and were excluded from the analysis (2 ovarian, 1 endocervix, 3 endometrial, 3 pancreatic, 2 primary liver cancer, 8 NSCLC, 2 SCLC, 1 breast, and 1 mesothelioma). Significantly higher serum concentrations of CA 125 and HE4 \( (P = 0.0001) \) were found in patients with cancer than in those with benign diseases, excluding renal failure. In malignant samples the median (SE) concentration of HE4 was 116.8 (25.4) pmol/L and of CA 125 was 44.2 (47.9) kU/L. The most common malignancy for which patients exhibited increased concentrations of HE4 and CA 125 was ovarian cancer (75.2% and 80% of samples, respectively). Significantly higher HE4 \( (P = 0.001) \) and CA 125 \( (P = 0.01) \) serum concentrations were found in patients with ovarian cancer than in patients with other gynecologic cancers \( (P = 0.001) \). Of the 275 samples from patients with nonovarian malignancies without renal failure, 49 exhibited increased HE4 and 122 exhibited increased CA 125. The most common malignancies in which patients had increased HE4 (using a cutoff of 140 pmol/L) were endometrial or endocervical adenocarcinomas, and lung cancer. By contrast, high concentrations of CA 125 were widely distributed, with abnormal concentrations seen in more than 40% of patients with nonovarian malignancies, including hematologic malignancies (Table 2). HE4 \( (P = 0.001) \), but not CA 125, was significantly higher in ovarian cancer than in NSCLC. HE4 in patients with malignancies other than ovarian cancer, endometrial cancer, or NSCLC was clearly related to the presence of liver metastases or effusions \( (P = 0.001) \). Modestly increased HE4 serum concentrations were seen in only 6.5% of patients with advanced malignancy and without liver metastases or effusions compared with 18.5% of patients with effusions or liver metastases. It is interesting to note that HE4 serum concentrations in patients with benign effusions or liver injury (Table 1) were similar to the concentrations found in patients with malignancies other than ovarian cancer, endometrial cancer, or NSCLC and liver metastases or effusions (Table 2).
Table 1. Serum concentrations of HE4 and CA 125 in healthy people and patients with benign diseases.

<table>
<thead>
<tr>
<th>Study participants, n</th>
<th>HE4 &gt; 140 pmol/L, n (%)</th>
<th>Median (SE), pmol/L</th>
<th>Range, pmol/L</th>
<th>95th Percentile, pmol/L</th>
<th>CA 125 &gt; 35 kU/L, n (%)</th>
<th>Median (SE), kU/L</th>
<th>Range, kU/L</th>
<th>95th percentile, kU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>101</td>
<td>1 (1)</td>
<td>53.4 (3.1)</td>
<td>13.9–184</td>
<td>121.7</td>
<td>10 (9.9)</td>
<td>5–69</td>
<td>44.8</td>
</tr>
<tr>
<td>Female</td>
<td>65</td>
<td>1 (1.5)</td>
<td>54 (4.1)</td>
<td>13.9–184</td>
<td>123</td>
<td>9 (13.8)</td>
<td>5–49</td>
<td>45.7</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>34</td>
<td>1 (2.9)</td>
<td>52.7 (6.5)</td>
<td>13.9–184</td>
<td>138.3</td>
<td>9 (28.5)</td>
<td>7–49</td>
<td>46.8</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>31</td>
<td>0 (0)</td>
<td>55.8 (5.1)</td>
<td>24.9–149</td>
<td>133.3</td>
<td>0 (0)</td>
<td>5–32</td>
<td>29</td>
</tr>
<tr>
<td>Male</td>
<td>36</td>
<td>0 (0)</td>
<td>49.2 (4.8)</td>
<td>15–122.3</td>
<td>117.2</td>
<td>1 (2.8)</td>
<td>10 (1.9)</td>
<td>5–69</td>
</tr>
<tr>
<td>Benign diseases</td>
<td>535</td>
<td>66 (12.3)</td>
<td>54 (5.7)</td>
<td>4.6–950</td>
<td>368.3</td>
<td>198 (37)</td>
<td>4–948</td>
<td>202</td>
</tr>
<tr>
<td>Renal failure or creatinine &gt; 1.3 mg/dL (&gt;115 μmol/L), no effusions</td>
<td>39</td>
<td>33 (84.6)</td>
<td>361.3 (42.5)</td>
<td>34.6–950</td>
<td>869.8</td>
<td>23 (59)</td>
<td>46 (15.6)</td>
<td>10–392</td>
</tr>
<tr>
<td>Effusions without renal failure [creatinine &gt; 1.3 mg/dL (&gt;115 μmol/L)]</td>
<td>59</td>
<td>21 (35.6)</td>
<td>119.4 (15.2)</td>
<td>22.2–451.9</td>
<td>434</td>
<td>47 (79.7)</td>
<td>120 (31.3)</td>
<td>7–948</td>
</tr>
<tr>
<td>Liver diseases without renal failure or effusions</td>
<td>44</td>
<td>2 (4.5)</td>
<td>81.1 (5.6)</td>
<td>33.5–176.1</td>
<td>150.1</td>
<td>20 (45.5)</td>
<td>32.4 (5.2)</td>
<td>6–207</td>
</tr>
<tr>
<td>No renal failure [creatinine &lt; 1.3 mg/dL (&lt;115 μmol/L)], effusion, or liver disease</td>
<td>29</td>
<td>3 (10.3)</td>
<td>71.6 (15)</td>
<td>24.3–411.2</td>
<td>334</td>
<td>7 (24.1)</td>
<td>19.4 (12.3)</td>
<td>7–352</td>
</tr>
<tr>
<td>Lung diseases</td>
<td>289</td>
<td>4 (1.3)</td>
<td>46.5 (1.7)</td>
<td>4.6–320.7</td>
<td>107</td>
<td>96 (33.2)</td>
<td>22 (2.4)</td>
<td>5–319</td>
</tr>
<tr>
<td>Gynecologic diseases</td>
<td>42</td>
<td>3 (7.1)</td>
<td>51.2 (6.5)</td>
<td>21.9–215.9</td>
<td>158.4</td>
<td>2 (4.8)</td>
<td>11 (1.6)</td>
<td>4–59</td>
</tr>
</tbody>
</table>

\( a P < 0.01. \)
\( b P = 0.005. \)
\( c P < 0.001. \)
\( d P = 0.023. \)
<table>
<thead>
<tr>
<th>HE4 &gt; 140 pmol/L, n (%)</th>
<th>Median (SE), pmol/L</th>
<th>Range, pmol/L</th>
<th>CA 125 &gt;25 kU/L, n (%)</th>
<th>Median (SE), kU/L</th>
<th>Range kU/L</th>
<th>95th percentile, kU/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total in all patients</td>
<td>160/423 (37.8)</td>
<td>121.2 (24.4)</td>
<td>16.1–7341</td>
<td>233/423 (55.1)</td>
<td>43 (45.4)</td>
<td>2–8630</td>
</tr>
<tr>
<td>Total in all patients without renal failure (creatinine &lt;1.3 mg/dL [&lt;115 μmol/L])</td>
<td>143/400 (35.6)</td>
<td>116.8 (25.4)</td>
<td>16.1–7341</td>
<td>222/400 (55.5)</td>
<td>44.2 (47.9)</td>
<td>2–8630</td>
</tr>
<tr>
<td>Totals according to malignancy in patients without renal failure</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary(^a)</td>
<td>94/125 (75.2)</td>
<td>357 (72.3)</td>
<td>17.2–7341</td>
<td>100/125 (80)</td>
<td>154 (107)</td>
<td>2–8630</td>
</tr>
<tr>
<td>Endometrium/endocervix(^a)</td>
<td>14/50 (28)</td>
<td>117.6 (14.1)</td>
<td>16.1–503.6</td>
<td>25/50 (50)</td>
<td>169 (313)</td>
<td>3–8340</td>
</tr>
<tr>
<td>Squamous cervical cancer(^a)</td>
<td>0/18 (0)</td>
<td>66.9 (7.6)</td>
<td>31–126</td>
<td>3/18 (16.7)</td>
<td>22.2 (35)</td>
<td>10–649</td>
</tr>
<tr>
<td>Breast(^a)</td>
<td>1/18 (5.6)</td>
<td>70.2 (9.5)</td>
<td>18.5–158</td>
<td>9/18 (50)</td>
<td>30 (25.6)</td>
<td>9–372</td>
</tr>
<tr>
<td>Digestive tract malignancies(^a)</td>
<td>6/53 (11.3)</td>
<td>80.3 (5.9)</td>
<td>23.6–192.2</td>
<td>22/53 (41.5)</td>
<td>26 (13.2)</td>
<td>4–510</td>
</tr>
<tr>
<td>Primary liver cancer(^a)</td>
<td>2/12 (16.3)</td>
<td>60.4 (28.8)</td>
<td>24.3–371.4</td>
<td>4/12 (33.3)</td>
<td>28.6 (4.4)</td>
<td>8–60</td>
</tr>
<tr>
<td>NSCLC(^a)</td>
<td>12/41 (29.3)</td>
<td>124.7 (27.4)</td>
<td>30.1–715.3</td>
<td>28/41 (68.3)</td>
<td>63.5 (238.2)</td>
<td>4–7449</td>
</tr>
<tr>
<td>SCLC(^a)</td>
<td>7/26 (26.9)</td>
<td>100.6 (31.1)</td>
<td>39.2–719.2</td>
<td>9/26 (34.6)</td>
<td>22.3 (9.8)</td>
<td>9–197</td>
</tr>
<tr>
<td>Urologic(^a)</td>
<td>3/14 (21.5)</td>
<td>97.5 (22.2)</td>
<td>52.4–375.4</td>
<td>5/14 (35.7)</td>
<td>20.5 (31.4)</td>
<td>4–457</td>
</tr>
<tr>
<td>Hematologic malignancies(^a)</td>
<td>3/30 (10)</td>
<td>80.5 (8.8)</td>
<td>26.3–236.1</td>
<td>14/30 (46.7)</td>
<td>31.5 (51.8)</td>
<td>9–1313</td>
</tr>
<tr>
<td>Melanoma(^a)</td>
<td>1/9 (11.1)</td>
<td>64 (15.7)</td>
<td>23.5–143.3</td>
<td>1/9 (10)</td>
<td>15.4 (4.2)</td>
<td>7–46.1</td>
</tr>
<tr>
<td>Mesenchymal tumors</td>
<td>0/4 (0)</td>
<td>83.9 (20.4)</td>
<td>33.4–129.8</td>
<td>2/4 (50)</td>
<td>41 (17.8)</td>
<td>11–85</td>
</tr>
<tr>
<td>Malignancies other than ovarian/endometrium/NSCLC without effusion or liver metastases(^b)</td>
<td>6/92 (6.5)</td>
<td>69.6 (4.5)</td>
<td>23.5–236.1</td>
<td>23/92 (25)</td>
<td>20.4 (14.5)</td>
<td>7–700</td>
</tr>
<tr>
<td>Malignancies other than ovarian/endometrium/NSCLC with effusion or liver metastases(^b)</td>
<td>17/92 (18.5)</td>
<td>86.8 (11)</td>
<td>18.5–719.2</td>
<td>46/92 (50)</td>
<td>35 (16.1)</td>
<td>5–1313</td>
</tr>
</tbody>
</table>

\(^a\) P = 0.001 with both tumor markers.

\(^b\) P = 0.001 with HE4.
The CA 125 and HE4 serum concentrations in patients with ovarian cancer, subdivided according to tumor stage and histological type, are shown in Table 3. Both tumor markers were clearly related to stage, with significantly higher concentrations in advanced stage III–IV than in stage I–II (CA 125: \( P = 0.001 \); HE4: \( P = 0.004 \)). However, no differences were found between stage III and IV with either tumor marker. The use of both tumor markers together improved the sensitivity obtained with only 1 tumor marker (Table 3). For patients with stage I–II cancer, HE4 was increased in 58.3%, CA 125 was increased in 54.2%, and 1 or both of the markers were increased in 70.8%. For patients with stage III–IV disease, 95% had increased concentrations of either marker, indicating that the use of HE4 together with CA 125 may improve the detection of ovarian cancer. Likewise, both tumor markers were related to the histological type, with significantly higher serum concentrations of CA 125 (\( P = 0.003 \)) and HE4 (\( P = 0.009 \)) in serous papillary ovarian cancer. No differences between mucinous tumors and other histologies (no serous-papillary) were found in relation to the serum concentrations of these tumor markers.

Fig. 1 A shows the ROC curve for evaluating the utility of HE4 and CA 125 in the diagnosis of malignancy (excluding those patients with renal failure or serum creatinine \( > 1.3 \text{ mg/dL} \) (\( > 115 \text{ µmol/L} \)), comparing patients with cancer and those with benign diseases. Fig. 1B shows the ROC curve, comparing the tumor marker utility in the diagnosis of ovarian cancer in relation to other benign or malignant diseases. HE4 showed a higher area under the curve in both situations. Fig. 2 shows the ROC curves comparing both tumor markers (A) in the diagnosis of gynecologic cancer vs benign gynecologic diseases and (B) in the diagnosis of ovarian cancer (ovarian cancer vs other gynecologic diseases). In all situations, HE4 showed a higher area under the curve than CA 125. Fig. 3 shows the ROC curves comparing both tumor markers in the diagnosis of ovarian cancer, according to menopausal status. HE4 had a higher area under the curve in both situations, but differences were not found to be statistically significant.

**Discussion**

Various authors have suggested the use of serial CA 125 in combination with ultrasonography in postmenopausal asymptomatic women as an aid in the early diagnosis of ovarian cancer (2, 3, 9, 25–28). Problems with this strategy are related to the limited tumor marker diagnostic sensitivity and specificity. These studies revealed a positive predictive value in the diagnosis of ovarian cancer in asymptomatic women from 10% to 21% (2–4, 9, 25–28). The major drawback of...
using CA 125 as an initial step in such a screening strategy is that up to 20% of ovarian cancers do not express the antigen (2–9, 24, 26, 28, 29). It is therefore necessary to combine CA 125 with new tumor markers that can provide better diagnostic efficiency (2, 3, 6–9, 25–29).

Diagnostic specificity is an important concern for CA 125, and abnormal values are seen in several benign and malignant diseases other than ovarian cancer (2–4, 7–16, 30–34). This lack of specificity is particularly important in some benign gynecologic conditions such as endometriosis, which is frequently diagnosed in premenopausal women. Despite these issues, CA 125 is still used to differentiate benign from malignant pelvic masses, and also as a prognostic factor for early diagnosis of recurrence and for assessment of response to treatment (2–4, 29).

The WAP four-disulfide core domain 2 (WFDC2) gene that encodes the HE4 protein is frequently overexpressed in ovarian cancers (21). In ovarian carcinomas, but not in normal tissue, the HE4 protein is N-glycosylated and secreted into the extracellular environment. Therefore, glycosylated HE4, with an apparent molecular weight of 25 kDa, may be secreted and become detectable in the bloodstream or urine of patients with ovarian carcinoma via a chemiluminescent enzyme immunoassay (22, 31). Tissue expression of HE4 has been reported to be increased in some pulmonary, endometrial, and breast adenocarcinomas, mesotheliomas, and less often, in gastrointestinal, renal, and transitional cell carcinomas (19–21).

Published results of studies of HE4 in serum indicate that HE4 diagnostic sensitivity and specificity in gynecologic diseases is better than that of CA 125 and that the tumor markers are complementary (7, 8, 17 33, 35–37). However, HE4 concentrations in patients with other benign or malignant nongynecologic diseases have not been characterized. Most tumor markers are increased in some benign conditions (2–4, 32, 34, 38). To avoid misinterpretations, the HE4 serum concentrations should be studied in different benign and malignant conditions.

Our results show that HE4 is less influenced by sex or menopausal status than CA 125 (39). HE4 was less
likely than CA 125 to be increased in benign conditions, including benign gynecologic conditions. HE4 was increased in 12.3% of patients with benign disease, and in only 1.3% of patients with gynecologic disease. In contrast, CA 125 was increased in 37% of patients with benign disease and in 33.2% of patients with gynecologic conditions. None of our 63 patients with endometriosis had abnormal HE4 serum concentrations in contrast to 49.2% with abnormal CA 125 concentrations. These results confirm previously published findings and clearly show that HE4 use may be important in the differential diagnosis of ovarian cancer from other gynecologic conditions, including those seen in premenopausal women (7, 8, 16–21, 35–37).

Renal failure is the most important source of false-positive HE4 results, similar to other tumor markers such as SCC (squamous cell carcinoma) antigen, S-100, and ProGRP (pro–gastrin-releasing peptide) (32, 34, 38).

The wide range of HE4 serum concentrations found in patients with renal failure clearly indicated that HE4 must be interpreted carefully in patients with renal failure or at least in those patients with abnormal creatinine serum concentrations. This is important, because acute renal failure may be found in some patients during chemotherapy.

The presence of effusions is a known source of false-positive results with CA 125 (4, 9, 10, 13–15). Abnormal serum concentrations of CA 125 were found in 79.7% of patients with effusions in this study. However, effusions had less influence on HE4 results than on CA 125, with 35.6% of patients with effusions exhibiting increased HE4. The majority of patients with effusions had HE4 ≥3 times the cutoff, contrasting with CA 125, for which serum concentrations may be 30 times higher than the cutoff. It is interesting to note that 10% of patients with benign lung diseases had small increases of HE4 (<500 pmol/L), and these data suggest that the presence of this antigen in normal lung epithelia, as has been demonstrated in lung adenocarcinomas and trachea or salivary gland (19, 20).

HE4 is less influenced by liver diseases, in which only small increases may be observed in fewer than 5% of patients, in contrast to the 24% rate seen with CA 125.
HE4 has been suggested as a tumor marker for ovarian cancer, and in our study, the highest diagnostic sensitivity and median concentrations of this antigen were found in ovarian cancer. Our results confirm those of previous studies of HE4 in gynecologic cancer, with minor differences in diagnostic sensitivity, possibly related to the patient characteristics (stage, histology) (7, 8). However, HE4 was not specific for ovarian cancer and abnormal concentrations of this tumor marker were also found in endometrial cancer, NSCLC, and primary liver cancer. Moore et al. (40) previously have noted the expression of HE4 in patients with endometrial cancer. Interestingly, none of the 18 patients with cervical squamous tumors had abnormal HE4 serum concentrations in our study. By contrast, one third of our patients with NSCLC, mainly adenocarcinomas, had increased concentrations, suggesting that this malignancy may produce HE4. No other reported studies have revealed this finding, but it seems reasonable in view of the presence of this antigen in lung adenocarcinoma tissues (19, 20).

The majority of patients with nonovarian or nonpulmonary malignancies with abnormal HE4 had only slightly high (<300 pmol/L) serum concentrations. Patients with advanced cancer and liver metastases or effusion had substantially higher serum HE4 than those without these metastases (Table 2). Likewise, the similar concentrations of HE4 observed in patients with benign or malignant effusion or liver injury (excluding ovarian cancer, endometrial cancer, and NSCLC) suggest that HE4 may reflect the presence of effusion or liver injury more than the release by the malignancy in these patients. In summary, HE4 specificity is substantially higher than CA 125 (see Fig. 1).

HE4 and CA 125 are related to tumor stage and histological type in ovarian cancer. However, it is interesting to note that these differences are mainly due to the lower concentrations of both tumor markers in stage I and II disease, with no significant differences between stages III and IV. CA 125 showed a higher sensitivity in advanced stages and similar or slightly higher HE4 sensitivity was obtained in early stages. The important point is that both tumor markers were complementary and their combined use increased the sensitivity obtained with either marker alone, primarily in stage I–II. However, the use of both tumor markers
HE4 and CA 125 in Benign and Malignant Diseases

decreased the specificity because the rate of CA 125 false positive results in gynecologic diseases is higher, mainly in premenopausal women. Abnormal CA 125 serum concentrations were found in 18.7% (54 patients) with benign disease, 52 of whom had HE4 serum concentrations were found in 18.7% (54 patients). Likewise, as previously reported, there was a relationship of both tumor markers to the histology, with significantly higher concentrations in serous-papillary malignancies (7, 8, 19, 20, 33). However, abnormal serum HE4 was found in 44% of mucinous tumors, and either or both tumor markers were abnormal in 75% of mucinous malignancies. Fig. 2 demonstrates the better HE4 diagnostic specificity and similar diagnostic sensitivity compared with CA 125, explaining the higher HE4 utility in the differential diagnosis of gynecologic diseases (7, 8, 19, 20, 33). This advantage of HE4 seems to be greater in premenopausal women (see Fig. 3), but the differences were not found to be statistically significant, and they may need to be confirmed in studies with higher numbers of patients.

In summary, HE4 is a new tumor marker useful in ovarian cancer. It shows a better specificity than CA 125 in benign, nongynecologic, and gynecologic diseases, as well as in the differential diagnosis of ovarian cancer from other malignant nonovarian diseases. Renal failure is the most important source of false-positive results with HE4, and HE4 results in patients with creatinine concentrations higher than 1.3 mg/dL (115 μmol/L) should be evaluated with caution. Abnormal serum concentrations of HE4 are mainly found in ovarian cancer, endometrial cancer, and NSCLC. HE4 improves the utility of CA 125 as a tumor marker in ovarian cancer, and using both markers simultaneously increases the tumor marker sensitivity in ovarian cancer.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

22. Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, Hecht JL. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is


