Human epididymis protein 4 (HE4) in benign and malignant diseases

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Abstract

Background: Human epididymis protein 4 (HE4) is described as a useful new biomarker in ovarian cancer. As HE4 is neither tumor nor organ specific, we intensively investigated the occurrence of this protein in female and male patients with various benign and malignant diseases in order to avoid misinterpretation and to identify potential additional clinical relevance.


Results: The lowest concentrations of HE4 were observed in healthy men (median 26.2 pmol/L) followed by healthy women (median 40.4 pmol/L). In benign diseases, highest HE4 concentrations were seen in both women and men with renal failure (women, median 1041 pmol/L; men, median 1368 pmol/L). In women, the highest HE4 levels in malignant diseases were observed in ovarian cancer (median 242 pmol/L), whereas the highest HE4 concentrations in men occurred in lung cancer (median 89.2 pmol/L). The area under the curve (AUC) of HE4 in women was highest in ovarian cancer and borderline tumors as compared to benign gynecological disorders (88.9%), with a sensitivity of 67.4% at 95% specificity. Also, significantly elevated concentrations of HE4 with reference to the respective group of benign diseases were observed in uterus corpus and breast cancer as well as in lung cancer for men and women.

Conclusions: HE4 has the highest relevance in ovarian cancer but can be elevated in a variety of benign and malignant diseases.

Keywords: benign diseases; HE4; malignant diseases; tumor marker.

Introduction

Ovarian cancer is the leading cause of death among gynecologic malignancies. Due to the lack of diagnostic tools for early detection of ovarian cancer, the vast majority of patients are diagnosed when they have an advanced stage of disease. Thus, there is a need to detect ovarian cancer when it is sufficiently small to be curable by existing therapies (1). Cancer antigen 125 (CA 125) in serum has a high sensitivity for ovarian cancer, but CA 125 levels are frequently elevated in women with benign gynecological diseases, resulting in a reduced specificity of this marker, especially in premenopausal women (2, 3). Therefore, there is a need for additional markers that can complement CA 125, both for early detection and differential diagnosis. One of the most promising new biomarkers is the human epididymis protein 4 (HE4). The HE4 gene encodes a WAP-type four disulphide core domain-containing protein with a presumptive role in natural immunity. HE4 gene expression is highest in normal human trachea and salivary gland, but also active in lung, prostate, pituitary gland, thyroid and kidney. Multiple studies have consistently identified an upregulation of HE4 gene expression in carcinomas of the ovary (4, 5). Within a relatively short time it became evident that HE4 is also released significantly into the blood by ovarian cancer and that this release offers an incremental diagnostic value in addition to CA 125 in ovarian cancer (6–16). Intense gene expression studies on HE4 revealed significant expression and strong immunoreactivity in ovarian cancer, but also in some pulmonary, endometrial and breast adenocarcinomas as well as gastrointestinal and urological carcinomas. Thus, HE4 like all other biomarkers is not a tumor specific protein and, like the vast majority of biomarkers, also not organ specific.

We performed this study to analyze the release of or of HE4 into the blood by various benign and malignant diseases to reduce misinterpretations when applying biomarkers in diagnostic oncology like in ovarian cancer diagnostics and other tumor diseases. Therefore, we investigated samples from female and male healthy individuals and patients with benign and malignant diseases.
Materials and methods

Controls and patients

We retrospectively evaluated the sera of 205 healthy individuals, 654 patients with various benign disorders and 720 patients suffering from cancer. The sera were collected between January 1986 and April 2009. All serum samples had been obtained at primary diagnosis and had been stored at –80°C. A plot of HE4 values against storage time of the samples revealed no dependencies.

All 205 healthy individuals underwent an intense anamnesis and were apparently healthy when the blood samples were drawn as well as 6 months later when consecutive blood samples were drawn. In addition, they had no suspicious findings in a broad profile of blood and urine analyses.

The 654 patients suffering from benign diseases were distributed as follows (Table 1): the group of benign lung diseases (n=50) consisted of bronchial asthma, bronchitis, chronic obstructive pulmonary disease (COPD), pneumonia, and benign lung tumors. Benign gastrointestinal diseases (n=82) mainly included hepatitis, cholangitis, choleliathiasis, cholestasis, liver cirrhosis, pancreatitis and liver adenomas. For benign urological diseases, patients with benign hyperplasia of the prostate (n=21) as well as patients with renal failure (n=33) were considered separately. The remaining benign urological diseases (n=24) included renal cysts, voiding problems, hydrenephrosis, renal and urethral calculi and prostatitis. Table 2 shows the distribution of benign gynecological diseases (n=396) in more detail.

All 720 patients with cancer underwent surgery and were histologically or morphologically diagnosed. The corresponding blood samples had been drawn before any treatment. The study was approved by the Local Ethical Committee. Informed consent was obtained from all patients participating in this study. In this retrospective evaluation, tumor marker levels were analyzed with reference to patient characteristics and clinical data.

Serum analysis

HE4 was analyzed using the ARCHITECT® system (Abbott Diagnostics, USA). The ARCHITECT assay for HE4 is a two-step immunoassay. In the first step, sample and 2H5 anti-HE4 coated paramagnetic microparticles are combined. HE4 antigen then binds to the anti-HE4 coated microparticles. In the second step, 3D8 anti-HE4 acridinium labeled conjugate is added to the HE4 assay. Chemiluminiscent reaction is measured in relative light units and directly reflects HE4 concentrations in the serum samples. The limit of detection (LoD) of this assay is 0.3 pmol/L, and the limit of quantitation (LoQ) is 1.4 pmol/L. The within-run imprecision of the ARCHITECT HE4 assay for three controls and three pools covering the measuring range up to 1500 pmol/L varies between 3.1% and 4.9% and the inter-assay imprecision between 4.5% and 5.1% (as indicated by the supplier).

Statistics

Statistical analysis was performed using SAS V9.2 (SAS Institute Inc., Cary, NC, USA). HE4 marker results are given as median, range and percentiles. HE4 concentrations were compared between groups using the Wilcoxon test. Dependency on age was tested using the general linear model (GLM) procedure for an overall p-value and the Ryan-Einot-Gabriel-Welsch multiple range test for assessing significant differences between the age groups. Receiver operating characteristic (ROC) curves were assessed to reflect the relationship between sensitivity and specificity for HE4. They were compared by means of the areas under the curves (AUC) and additionally by sensitivities at a set specificity of 95%.

For showing extreme values of HE4 we defined the upper limit of the healthy population as three times the interquartile range and added this value to the upper quartiles of the distribution stratified for gender and age (<50 and ≥50 years). When applying this rule, the extreme values reached for women were 96 pmol/L for <50 and 120 pmol/L for ≥50 years, for men 81 pmol/L <50 and 93 pmol/L ≥50 years. Values above the upper limit in the respective age and gender group are plotted in decreasing order of HE4 values.

Results

HE4 in healthy individuals

The group of healthy individuals consisted of 109 women and 96 men.

Figure 1A demonstrates the HE4 concentrations of healthy women by age decades. The median age of healthy women was 38.4 years (range 21.5–80.0 years). Median values of HE4 increased with age from 33.5 pmol/L in women under
our study.

we analyzed HE4 levels for men and women separately in

concentrations in men were significant lower than in women,

than 50 years, as well as between the group of men between

the group of men over 60 years and the groups of men younger

50 years and men younger than 30 years. As HE4 concen-

trations in men were significantly lower than in women, we

analyzed HE4 levels for men and women separately in

study.

HE4 in benign and malignant diseases

The distribution of HE4 values in women and men with

benign diseases is described in Table 1.

Both in women and men, the highest HE4 values were

observed in patients with renal failure. With the exception of

renal failure, median levels of HE4 in women with benign
diseases were comparable to the median of healthy women,

however with a broad range of values especially for benign
gastrointestinal and benign gynecological diseases. In benign
gastrointestinal diseases, highest HE4 values were seen in

liver cirrhosis and cholangitis. In benign gynecological dis-

eases, the highest single values were observed for women

with leiomyoma, while women with inflammatory diseases

and bleeding disorders had the highest median values of HE4

(Table 2).

The group of bleeding disorders includes 12 cases of post-

menopausal bleeding, six women with menometrorrhagia,

five cases of perimenopausal bleeding, two patients suffering

dysmenorrhea, one woman with serometra, one patient

with endometrial polyp, one case of hematomas in uterus

duplex, one case of suspicious endometrium, two cases of

endometrial hyperplasia and one bleeding disorder of a patient

with a persistent high β-HCG value. Except for one woman

with dysmenorrhea, all patients received a hysteroscopy and

ablation with histopathological analysis. All cases showed

benign results. Low HE4 values in the subgroup of benign
gynecological diseases were observed for endometriosis,
followed by benign cervix diseases. Lowest concentrations
of HE4 in women were observed in benign breast disorders

(Table 1).

In men, all benign disease groups had higher median HE4

values and broader ranges than healthy men and the corre-
sponding female disease group (Table 1). Similar to women,

high values in benign gastrointestinal diseases were observed

in liver cirrhosis and cholestasis. In benign urological dis-

eases, highest HE4 values were seen for urethral calculus

and prostatitis, in benign lung diseases for pneumonia and

benign tumors.

Table 3 lists HE4 values of women and men with different

malignant diseases. Highest HE4 concentrations in women

were observed in ovarian cancer (p vs. benign gynecological

diseases: <0.0001). Significantly higher values than in

the respective organ-related control group of benign lung dis-

eases were also observed for lung cancer (p=0.0001), as well as

for borderline tumors (p=0.008) and uterus corpus cancer

(p=0.001) as compared to the organ-related control group of

benign gynecological diseases. Also, breast cancer patients

showed higher HE4 concentrations than the respective organ-

related control group of benign breast diseases (p=0.008).

Table 1  Distribution of HE4 in healthy controls and patients affected by benign diseases.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gender</th>
<th>n</th>
<th>Age, years</th>
<th>HE4, pmol/L</th>
<th>Pctl95</th>
<th>p vs. healthy</th>
<th>p vs. women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median</td>
<td>Median</td>
<td>Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td>Females</td>
<td>109</td>
<td>38.4</td>
<td>40.4</td>
<td>10.0–111</td>
<td>72.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>96</td>
<td>37.2</td>
<td>26.2</td>
<td>13.6–87.2</td>
<td>46.9</td>
<td></td>
</tr>
<tr>
<td>Benign lung disease</td>
<td>Females</td>
<td>19</td>
<td>59.9</td>
<td>44.4</td>
<td>25.5–107</td>
<td>107</td>
<td>0.174</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>31</td>
<td>59.3</td>
<td>57.1</td>
<td>20.3–237</td>
<td>170</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Benign gastrointestinal disease</td>
<td>Females</td>
<td>44</td>
<td>54.1</td>
<td>36.5</td>
<td>16.4–424</td>
<td>289</td>
<td>0.941</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>Males</td>
<td>38</td>
<td>50.3</td>
<td>53.7</td>
<td>20.6–2366</td>
<td>989</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>16</td>
<td>50.1</td>
<td>1042</td>
<td>53.4–2321</td>
<td>2321</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other benign urological disease</td>
<td>Females</td>
<td>6</td>
<td>62.0</td>
<td>1368</td>
<td>28.4–7043</td>
<td>7043</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>18</td>
<td>56.2</td>
<td>34.3</td>
<td>25.9–50.4</td>
<td>50.4</td>
<td>0.225</td>
</tr>
<tr>
<td>Benign prostate hyperplasia</td>
<td>Males</td>
<td>21</td>
<td>70.3</td>
<td>43.1</td>
<td>22.8–263</td>
<td>263</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Benign breast disease</td>
<td>Females</td>
<td>48</td>
<td>44.9</td>
<td>48.4</td>
<td>24.2–710</td>
<td>235</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Benign gynecological disease</td>
<td>Females</td>
<td>396</td>
<td>44.8</td>
<td>31.7</td>
<td>16.4–81.1</td>
<td>63.7</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Table 2  Distribution of HE4 in benign gynecological diseases.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n</th>
<th>Age, years</th>
<th>HE4, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Median</td>
</tr>
<tr>
<td>Benign cervix dis.</td>
<td>36</td>
<td>37.5</td>
<td>38.8</td>
</tr>
<tr>
<td>Cystadenoma</td>
<td>78</td>
<td>54.2</td>
<td>42.8</td>
</tr>
<tr>
<td>Leiomyoma</td>
<td>66</td>
<td>43.1</td>
<td>39.3</td>
</tr>
<tr>
<td>Bleeding disorder</td>
<td>32</td>
<td>51.1</td>
<td>43.4</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>52</td>
<td>35.6</td>
<td>36.3</td>
</tr>
<tr>
<td>Functional ovarian cysts</td>
<td>84</td>
<td>44.8</td>
<td>41.1</td>
</tr>
<tr>
<td>Inflammatory disease</td>
<td>10</td>
<td>44.0</td>
<td>67.6</td>
</tr>
<tr>
<td>Other benign gynecological diseases</td>
<td>38</td>
<td>46.6</td>
<td>38.7</td>
</tr>
</tbody>
</table>

Table 3  Distribution of HE4 in patients affected by malignant diseases.

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Gender</th>
<th>n</th>
<th>Age, years</th>
<th>HE4, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median</td>
<td>Median</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>Females</td>
<td>23</td>
<td>61.2</td>
<td>77.3</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>77</td>
<td>62.8</td>
<td>89.2</td>
</tr>
<tr>
<td>Ear nose throat cancer</td>
<td>Females</td>
<td>4</td>
<td>58.6</td>
<td>51.2</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>26</td>
<td>58.7</td>
<td>68.4</td>
</tr>
<tr>
<td>Gastric cancer</td>
<td>Females</td>
<td>24</td>
<td>67.2</td>
<td>46.2</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>27</td>
<td>59.7</td>
<td>48.6</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>Females</td>
<td>12</td>
<td>65.6</td>
<td>64.4</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>27</td>
<td>66.0</td>
<td>62.0</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Females</td>
<td>20</td>
<td>63.6</td>
<td>42.9</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>30</td>
<td>63.2</td>
<td>60.4</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Females</td>
<td>21</td>
<td>62.6</td>
<td>47.0</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>31</td>
<td>64.2</td>
<td>54.2</td>
</tr>
<tr>
<td>Esophagus/anal cancer</td>
<td>Females</td>
<td>16</td>
<td>72.7</td>
<td>42.1</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>19</td>
<td>63.5</td>
<td>66.1</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>Females</td>
<td>9</td>
<td>70.3</td>
<td>67.8</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>33</td>
<td>61.3</td>
<td>73.5</td>
</tr>
<tr>
<td>Renal cancer</td>
<td>Females</td>
<td>16</td>
<td>66.3</td>
<td>40.6</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>24</td>
<td>62.3</td>
<td>59.1</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Males</td>
<td>30</td>
<td>61.3</td>
<td>39.8</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Females</td>
<td>50</td>
<td>53.1</td>
<td>37.2</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>Females</td>
<td>125</td>
<td>62.9</td>
<td>242</td>
</tr>
<tr>
<td>Borderline tumor</td>
<td>Females</td>
<td>16</td>
<td>59.7</td>
<td>52.1</td>
</tr>
<tr>
<td>Cervix cancer</td>
<td>Females</td>
<td>41</td>
<td>54.1</td>
<td>43.3</td>
</tr>
<tr>
<td>Uterus corpus cancer</td>
<td>Females</td>
<td>19</td>
<td>62.8</td>
<td>53.7</td>
</tr>
</tbody>
</table>

Table 4  Distribution of HE4 in dependence of the histological type in ovarian cancer.

<table>
<thead>
<tr>
<th>Histology</th>
<th>n</th>
<th>Age, years</th>
<th>HE4, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median</td>
<td>Median</td>
</tr>
<tr>
<td>Serous</td>
<td>84</td>
<td>62.9</td>
<td>386</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>12</td>
<td>63.2</td>
<td>165</td>
</tr>
<tr>
<td>Mucinous</td>
<td>8</td>
<td>67.7</td>
<td>74.1</td>
</tr>
<tr>
<td>Other</td>
<td>13</td>
<td>55.4</td>
<td>59.5</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>8</td>
<td>73.6</td>
<td>327</td>
</tr>
</tbody>
</table>
In men, the highest HE4 levels were seen in lung cancer (p vs. benign lung diseases 0.002) (Table 3).

Figure 2 illustrates high HE4 values separately for sex and age groups. With the exception of women over 50, where the highest values were seen for ovarian cancer, patients with renal failure generally reached highest HE4 levels. Apart from renal insufficiency and ovarian cancer, in the group of young women high HE4 values were seen for one patient with lung cancer and nine patients with benign gynecological, gastrointestinal and lung diseases. In this group of patients, women with myoma and cholangitis reached the highest HE4 values (Figure 2A).

In women over 50 years of age 28 patients with carcinoma (6 gynecological, 8 gastrointestinal, 5 lung, 5 breast and 4 other) and also 12 patients with benign gynecological and gastrointestinal diseases showed extremely high HE4 values. This was mainly caused by patients with myoma and liver cirrhosis.

As in women, also in the older age group of men malignant diseases showed high HE4 values (31 lung cancers, 22 gastrointestinal cancers, and 24 other cancers), whereas in the younger age group highest values were observed in liver cirrhosis.

Table 5 shows AUCs and sensitivities of HE4 at for malignant diseases 95% specificity in women and men. A significant difference in HE4 values is detected compared to the respective benign disease. The largest area under the curve of HE4 in women was seen for the differentiation of ovarian cancer and borderline tumors from benign gynecological diseases, amounting to 88.9%. Even if healthy individuals and all benign and malignant diseases except ovarian cancer and borderline tumors served as one control group as compared to ovarian cancer and borderline tumors as disease group, the sensitivity of HE4 was still 55.3% at 95% specificity and the corresponding AUC reached 86.2% (Figure 3).

In men, only lung cancer showed significantly higher HE4 values than benign lung diseases with an AUC of 68.9% and a sensitivity of 11.7% (Table 5).

Figure 2  Extreme values of HE4 for women (A) and men (B). The upper curve is related to patients aged ≥50 years.

Discussion

Ovarian cancer is often detected at an advanced stage and thus ranks as the fifth most common cause of death in women (17). Besides vaginal sonography, tumor marker values may aid to detect ovarian cancer in early stages. For almost 30 years, the tumor marker CA 125 was the most relevant biomarker for the management of patients with ovarian cancer (18). However, for the purpose of primary diagnosis, in clinical practice corresponding to the differential diagnosis of pelvic masses, the use of CA 125 has its limitations. The reason is not a lack of sensitivity of CA 125 for ovarian cancer, but the varying individual baseline values of CA 125 in healthy women. The lack of specificity is caused by frequently increased levels of CA 125 in benign gynecological diseases such as endometriosis, fibromas, uterine myomas, acute salpingitis and pelvic inflammatory diseases (19). In addition, several non-gynecological malignant diseases like lymphoma, lung cancer, breast cancer.
and gastrointestinal cancers may release CA 125 as well as benign diseases affecting the serous membranes of the peritoneum (liver cirrhosis, chronic active hepatitis, pancreatitis), pleura or pericardium (2, 19). Therefore, further biomarkers are needed for the early detection and differential diagnosis of ovarian cancer. HE4 is one of the latest, most promising biomarkers in this field (5). First studies concerning HE4 in blood indicate this protein to be frequently released by ovarian cancer. In addition, HE4 offers a superior specificity in benign gynecological diseases as compared to CA 125 (11, 20, 21). In literature, the combination of both biomarkers has been shown an increased specificity and sensitivity in the differential diagnosis of pelvic masses (7, 8, 13–16).

As systemic blood serum levels are not related to a specific organ in contrary to clinical examination, medical imaging or tissue analysis, it is important to investigate the level of HE4 in the blood of healthy individuals and especially in patients with any non-gynecological benign diseases or other malignant diseases besides ovarian cancer. Our investigation considers women and men, in order to be able to observe hormonal relations and potentially clinically relevant releases in any other cancer.

Our first obvious finding was the dependency of HE4 values from gender and age. In healthy individuals, HE4 values for men (median 26.2 pmol/L) were clearly lower than values for women (median 40.4 pmol/L). In both sexes, the HE4 level increased with age. In regard to this finding, age and gender specific reference ranges will have to be considered.

In benign diseases, highest HE4 values for both sexes were seen in patients with renal failure (women, median 1042 pmol/L; men, median 1368 pmol/L). This result is similar to other biomarkers like ProGRP or SCCA and was also described recently by Escudero 2011 for HE4 (12). As a consequence, HE4 should not be used in patients with renal insufficiency or patients with abnormal serum creatinine levels. This finding can especially become relevant in patients receiving nephrotoxic chemotherapy who may develop renal disorders over time.

The majority of benign gastrointestinal diseases were not associated with an increase of HE4, which is different from the experience with CA 125. Nevertheless, especially in patients with liver cirrhosis and especially in men, HE4 reached high serum values up to 2000 pmol/L. However, liver cirrhosis is not frequent in women and therefore rarely problematic in the differential diagnosis of ovarian cancer. Patients with chronic disease like liver cirrhosis frequently show higher biomarker levels for HE4 as it is also known for α-fetoprotein (AFP), CA 125, carcinoembryonic antigen (CEA) and CA 19-9 due to the impaired liver metabolism.

Somewhat higher HE4 values (up to 230 pmol/L) are also being observed in benign lung diseases, especially in men. This release arises most probably from the physiological expression of HE4 in the trachea, salivary glands and pulmonary tissue as described earlier.

In the analysis of the different benign gynecological diseases, HE4 values for women with endometriosis were in the range of healthy women (median 36.3 pmol/L). This is an important finding since endometriosis frequently entails high levels of CA 125 (10, 22). If the analysis of HE4 in these patients shows low values, malignancy is unlikely.

In our study, we observed the highest release of HE4 among malignant diseases in ovarian cancer (median 242 pmol/L, maximum 7507 pmol/L). The histological subtypes of ovarian cancer showed a varying HE4 release which was highest in the serous subtype (median 386 pmol/L, range 31.6–7507 pmol/L) and lowest in the mucinous subtype (median 74.1 pmol/L, range 35.4–447 pmol/L). Our data are consistent with the oligonucleotide microarray analysis of HE4 in malignant ovarian tissue by Drapkin et al. (5) and Galgano et al. (4). In those studies the highest staining for HE4 was seen in serous ovarian carcinomas, whereas mucinous ovarian carcinomas showed negative staining for HE4. This high release in serous ovarian cancer and only slightly increased release in mucinous ovarian cancer is also known for CA 125. Thus, both biomarkers do not offer a relevant diagnostic capacity for mucinous ovarian cancer, neither alone or in combination.

With 77 pmol/L for women and 89 pmol/L for men, the second highest median HE4 values in women and men among the malignant diseases were seen in lung cancer patients. However, the sensitivity for the differentiation of lung cancer from benign lung disease was low in both sexes (women 26%,

Figure 3 ROC curve for HE4 ARCHITECT in women.
(A) Ovarian cancer and borderline tumor of the ovary vs. benign gynecological diseases. (B) Ovarian cancer and borderline tumor of the ovary vs. all other diseases.
men 12%). Nevertheless, it remains to be analyzed whether this release of HE4 in lung cancer patients is potentially additive to the established biomarkers CEA, Serum Cytokeratin Fragment (CYFRA) 21-1, SCCA, ProGRP and Neuron Specific Enolase (NSE). In elderly women and men, a broad variety of malignant tumor diseases lead to clearly elevated HE4 levels. In contrast younger women (apart from ovarian cancer) and men with benign diseases present with low HE4 values.

In summary, our data confirm HE4 in blood as a non-tumor specific and non-organ specific biomarker. Our results also confirm by far the highest release of this protein in ovarian cancer if patients with renal failure are excluded from the investigation.

The AUC of HE4 in women in the differentiation of ovarian cancer and borderline tumors from healthy individuals and all patients affected by other benign and malignant diseases was very high, amounting to 86.2%. Almost the same area under the curve was observed in the study of Escudero et al. (86.6%) (12). As a consequence, very high HE4 levels are able to confirm the diagnosis of ovarian cancer.

Especially in clinical differential diagnosis of ovarian masses, it is of interest as to whether the early ovarian tumor stages International Federation of Gynecologists and Obstetricians (FIGO) I and II are present with elevated HE4 values. In younger women (<50 years), the patients with ovarian cancer FIGO II had the highest HE4 value. All other ovarian cancer patients with extremely high values in this age group had ovarian cancer stage FIGO III. In elderly women (>50 years), nine women with very high HE4 values presented with stage FIGO I, and also nine with FIGO stage II. Among 60 of these female patients >50 years, only two showed HE4 values >300 pmol/L and suffered from a benign disease. All other cases were cancer patients, among them 51 with ovarian cancer.

Ovarian cancer patients who present with clinical symptoms are frequently diagnosed with late stage disease. Since early tumor stage is associated with a better prognosis in ovarian cancer patients, detection at an early tumor stage is crucial. Due to the high specificity of this biomarker for ovarian cancer and the high prevalence of elevated HE4 in early stage of this disease, it gives rise to the hope that HE4 can be helpful to detect ovarian cancer in an asymptomatic, early tumor stage.

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Conflict of interest statement

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