Clin Chem Lab Med 2012;50(12):2181–2188 © 2012 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/cclm-2012-0097

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

Linda Hertlein[1,*], Petra Stieber[2], Angela Kirschenhofer[1], Sophie Fürst[1], Doris Mayr[3], Karin Hofmann[2], Katja Krocker[2], Dorothea Nagel[2], Miriam Lenhard[1,a] and Alexander Burges[1,a]

[1] Department of Obstetrics and Gynecology, University of Munich, Campus Grosshadern, Munich, Germany
[2] Department of Clinical Chemistry, University of Munich, Campus Grosshadern, Munich, Germany
[3] Department of Pathology, University of Munich, Campus Grosshadern, Munich, Germany

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.
Methods: We retrospectively investigated HE4 (ARCHITECT®, Abbott Diagnostics, US) in the sera of 205 healthy individuals, 654 patients with benign disorders and 720 patients with cancer before initial treatment.
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 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.

[a] Miriam Lenhard and Alexander Burges contributed equally to this manuscript.

*Corresponding author: Linda Hertlein, MD, Department of Obstetrics and Gynecology Ludwig-Maximilians-University of Munich, Campus Grosshadern, Marchioninistrasse 15, 81377 Munich, Germany
Phone: +49 89 70950, Fax: +49 89 70955844,
E-mail: Linda.Hertlein@med.uni-muenchen.de

Received February 14, 2012; accepted June 8, 2012; previously published online July 24, 2012

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, cholelithiasis, 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, hydronephrosis, renal and uretheral 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 histomorphologically 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

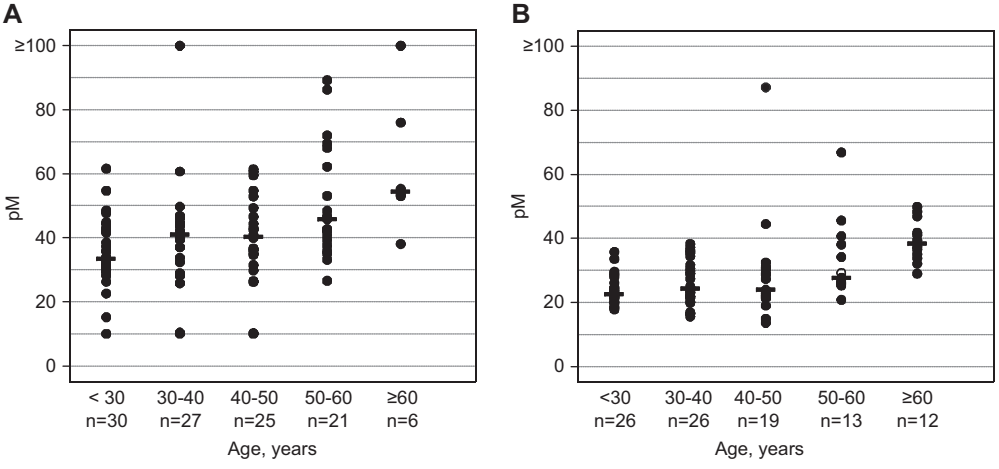

Figure 1 Distribution of HE4 values in healthy women (A) and men (B).

30 years to 54.5 pmol/L in women over 60 years (overall p<0.001). Differences were significant between the group of women over 60 years and all other groups as well as between the group of women between 50 and 60 years and women younger than 30 years.

The median age of healthy men was 37.2 years (range 21.5–80.0 years) and thus comparable to the age of healthy women (p=0.811). Median HE4 concentrations in healthy men also increased with age (Figure 1B) from 22.6 pmol/L in men under 30 years to 38.4 pmol/L in men over 60 years (overall p<0.001). Differences were significant between the group of men over 60 years and the groups of men younger than 50 years, as well as between the group of men between 50 and 60 years and men younger than 30 years. As HE4 concentrations in men were significantly lower than in women, we analyzed HE4 levels for men and women separately in our 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 diseases, 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 postmenopausal bleeding, six women with menometrorrhagia, five cases of perimenopausal bleeding, two patients suffering from dysmenorrhea, one woman with serometra, one patient with endometrial polyp, one case of hematocolpos 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 abrasion with histomorphological 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 corresponding female disease group (Table 1). Similar to women, high values in benign gastrointestinal diseases were observed in liver cirrhosis and cholestasis. In benign urological diseases, highest HE4 values were seen for uretheral 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 diseases 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.

Group	Gender	n	Age, years	HE4, pmol/L				
			Median	Median	Range	Pctl95	p vs. healthy	p vs. women
Healthy	Females	109	38.4	40.4	10.0–111	72.0		
	Males	96	37.2	26.2	13.6–87.2	46.9		<0.001
Benign lung disease	Females	19	59.9	44.4	25.5–107	107	0.174	
	Males	31	59.3	57.1	20.3–237	170	<0.001	0.089
Benign gastrointestinal disease	Females	44	54.1	36.5	16.4–424	289	0.941	
	Males	38	50.3	53.7	20.6–2366	989	<0.001	0.003
Renal insufficiency	Females	16	50.1	1042	53.4–2321	2321	<0.001	
	Males	17	62.0	1368	28.4–7043	7043	<0.001	0.048
Other benign urological disease	Females	6	62.5	34.3	25.9–50.4	50.4	0.225	
	Males	18	56.2	43.1	22.8–263	263	<0.001	0.110
Benign prostate hyperplasia	Males	21	70.3	48.4	24.2–710	235	<0.001	
Benign breast disease	Females	48	44.9	31.7	16.4–81.1	63.7	0.002	
Benign gynecological disease	Females	396	44.8	40.7	18.8–1178	94.6	0.297	

Table 2 Distribution of HE4 in benign gynecological diseases.

Diagnosis	n	Age, years	HE4, pmol/L			
		Median	Median	Range	Pctl5	Pctl95
Benign cervix dis.	36	37.5	38.8	22.1–177	24.8	125
Cystadenoma	78	54.2	42.8	20.4–145	27.5	94.6
Leiomyoma	66	43.1	39.3	20.1–1178	24.8	102
Bleeding disorder	32	51.1	43.4	25.2–112	26.8	82.3
Endometriosis	52	35.6	36.3	20.3–133	26.3	74.6
Functional ovarian cysts	84	44.8	41.1	18.8–194	24.2	88.0
Inflammatory disease	10	44.0	67.6	39.9–120	39.9	120
Other benign gynecological diseases	38	46.6	38.7	26.6–277	27.7	130

Table 4 shows HE4 values in the different histological subgroups of ovarian cancer. The highest HE4 release was seen in the serous type (median 386 pmol/L, range 31.6–7507 pmol/L) followed by the undifferentiated type (median 327.5, range 146–1009 pmol/L). Mucinous ovarian cancers had the lowest HE4 values (median 74.1, range 35.4–447 pmol/L). The group of others in Table 4 included five solid, low differentiated carcinomas of the ovary, three granulosa cell tumors, three malignant mixed müllerian tumors, one sarcoma of the ovary, one neuroendorendocrine tumor of the ovary and one clear cell carcinoma of the ovary.

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

Histology	n	Age, years	HE4, pmol/L			
		Median	Median	Range	Pctl5	Pctl95
Serous	84	62.9	386	31.6–7507	54.2	3124
Endometroid	12	63.2	165	39.7–3469	39.7	3469
Mucinous	8	67.7	74.1	35.4–447	35.4	447
Other	13	55.4	59.5	29.0–2301	29.0	2301
Undifferentiated	8	73.6	327	146–1009	146	1009

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

Cancer	Gender	n	Age, years	HE4, pmol/L				
			Median	Median	Range	Pctl95	p vs. healthy	p vs. women
Lung cancer	Females	23	61.2	77.3	42.0–1529	710	<0.001	
	Males	77	62.8	89.2	38.4–2126	322	<0.001	0.594
Ear nose throat cancer	Females	4	58.6	51.2	25.4–1780	1780	0.675	
	Males	26	58.7	68.4	30.5–229	146	<0.001	0.542
Gastric cancer	Females	24	67.2	46.2	27.0–523	288	<0.001	
	Males	27	59.7	48.6	23.9–2482	126	0.018	0.756
Liver cancer	Females	12	65.6	64.4	30.3–217	217	<0.001	
	Males	27	66.0	62.0	28.6–439	299	<0.001	0.952
Pancreatic cancer	Females	20	63.6	42.9	25.0–146	134	<0.001	
	Males	30	63.2	60.4	25.7–396	259	<0.001	0.231
Colorectal cancer	Females	21	62.6	47.0	27.2–112	96.7	<0.001	
	Males	31	64.2	54.2	22.8–129	124	0.027	0.751
Esophagus/anal cancer	Females	16	72.7	42.1	22.6–223	223	<0.001	
	Males	19	63.5	66.1	25.7–1487	1487	0.423	0.094
Bladder cancer	Females	9	70.3	67.8	34.7–484	484	<0.001	
	Males	33	61.3	73.5	29.7–267	249	0.002	0.878
Renal cancer	Females	16	66.3	40.6	22.2–185	185	<0.001	
	Males	24	62.3	59.1	21.7–585	359	0.613	0.077
Prostate cancer	Males	30	61.3	39.8	17.1–132	72.2	0.002	
Breast cancer	Females	50	53.1	37.2	22.5–483	172	0.996	
Ovarian cancer	Females	125	62.9	242	29.0–7507	2954	<0.001	
Borderline tumor	Females	16	59.7	52.1	28.2–399	399	0.002	
Cervix cancer	Females	41	54.1	43.3	22.1–2412	138	0.110	
Uterus corpus cancer	Females	19	62.8	53.7	31.1–523	523	<0.001	

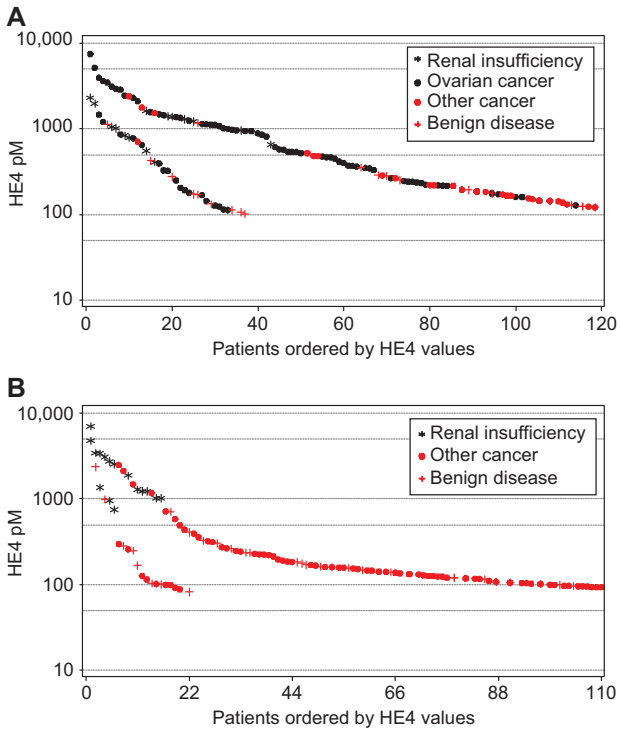

Figure 2 Extreme values of HE4 for women (A) and men (B). The upper curve is related to patients aged ≥50 years, the lower curve to patients under 50 years.

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).

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

Table 5 Sensitivity of HE4 ARCHITECT at 95% specificity and AUC values with lower confidence limits (LCL) and upper confidence limits (UCL) in the differentiation of benign and malignant diseases.

Cancer	Control	Gender	ND	NC	Sensitivity, %	AUC (LCL –UCL), %	p-Value
Lung cancer	Benign lung disease	Females	23	19	26.1	84.7 (72.8–96.5)	<0.001
		Males	77	31	11.7	68.9 (56.8–80.9	0.002
Breast cancer	Benign breast disease	Females	50	48	18.0	65.7 (54.9–76.5)	0.005
Ovarian cancer/Borderline tumor	Benign gynecological disease	Females	141	396	67.4	88.9 (85.3–92.5)	<0.001
Uterus corpus cancer	Benign gynecological disease	Females	19	396	21.1	72.1 (60.8–83.4)	<0.001
Ovarian cancer/borderline tumor	Healthy and all other benign and malignant diseases	Females	141	893	55.3	86.2 (82.6–89.8)	<0.001

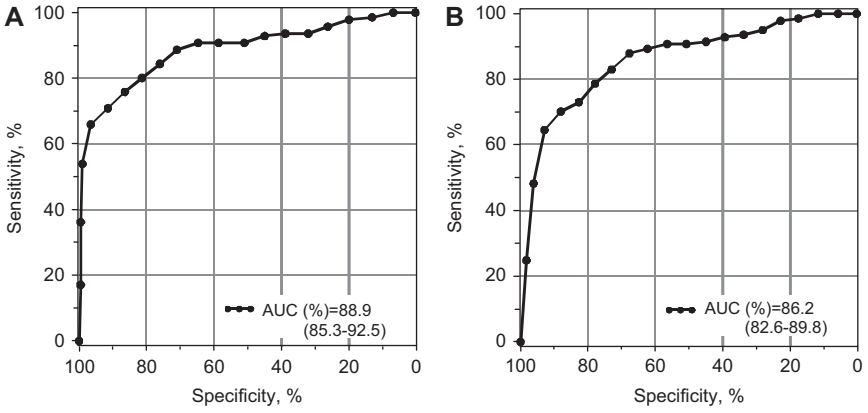

Figure 3 ROC curve for HE4 ARCHITECT in women.
(A) Ovarian cancer and borderline tumor of the ovary vs. benign gynecological diseases. (B) Ovarian cancer and borderline tumor of the ovary vs. all other diseases.

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%,

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.

Acknowledgments

We thank Dr. Barry Dowell and Sabrina Chan (Abbott Diagnostics, USA) for their support with reagents and equipment and for contributing their scientific know-how in data evaluation and discussion.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

References

1. Kurman RJ, Visvanathan K, Roden R, Wu TC, Shih Ie M. Early detection and treatment of ovarian cancer: shifting from early stage to minimal volume of disease based on a new model of carcinogenesis. Am J Obstet Gynecol 2008;198:351–6.
2. Jacobs I, Bast RC Jr. The CA 125 tumour-associated antigen: a review of the literature. Hum Reprod 1989;4:1–12.
3. Bast RC Jr, Badgwell D, Lu Z, Marquez R, Rosen D, Liu J, et al. New tumor markers: CA125 and beyond. Int J Gynecol Cancer 2005;15(Suppl 3):274–81.
4. Galgano MT, Hampton GM, Frierson HF Jr. Comprehensive analysis of HE4 expression in normal and malignant human tissues. Mod Pathol 2006;19:847–53.
5. Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res 2005;65:2162–9.
6. Hellstrom I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res 2003;63:3695–700.
7. Moore RG, McMeekin DS, Brown AK, DiSilvestro P, Miller MC, Allard WJ, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecol Oncol 2009;112:40–6.
8. Hellstrom I, Hellstrom KE. SMRP and HE4 as biomarkers for ovarian carcinoma when used alone and in combination with CA125 and/or each other. Adv Exp Med Biol 2008;622:15–21.
9. Anastasi E, Marchei GG, Viggiani V, Gennarini G, Frati L, Reale MG. HE4: a new potential early biomarker for the recurrence of ovarian cancer. Tumour Biol 2010;31:113–9.
10. Huhtinen K, Suvitie P, Hiissa J, Junnila J, Huvila J, Kujari H, et al. Serum HE4 concentration differentiates malignant ovarian tumours from ovarian endometriotic cysts. Br J Cancer 2009;100:1315–9.
11. Montagnana M, Lippi G, Ruzzenente O, Bresciani V, Danese E, Scevarolli S, et al. The utility of serum human epididymis protein 4 (HE4) in patients with a pelvic mass. J Clin Lab Anal 2009;23:331–5.
12. Escudero JM, Auge JM, Filella X, Torne A, Pahisa J, Molina R. Comparison of serum human epididymis protein 4 with cancer antigen 125 as a tumor marker in patients with malignant and nonmalignant diseases. Clin Chem 2011;57:1534–44.
13. Lenhard M, Stieber P, Hertlein L, Kirschenhofer A, Furst S, Mayr D, et al. The diagnostic accuracy of two human epididymis protein 4 (HE4) testing systems in combination with CA125 in the differential diagnosis of ovarian masses. Clin Chem Lab Med 2011;49:2081–8.
14. Park Y, Lee JH, Hong DJ, Lee EY, Kim HS. Diagnostic performances of HE4 and CA125 for the detection of ovarian cancer from patients with various gynecologic and non-gynecologic diseases. Clin Biochem 2011;44:884–8.
15. Andersen MR, Goff BA, Lowe KA, Scholler N, Bergan L, Drescher CW, et al. Use of a symptom index, CA125, and HE4 to predict ovarian cancer. Gynecol Oncol 2010;116:378–83.
16. Nolen B, Velikokhatnaya L, Marrangoni A, De Geest K, Lomakin A, Bast RC Jr, et al. Serum biomarker panels for the

discrimination of benign from malignant cases in patients with an adnexal mass. Gynecol Oncol 2010;117:440–5.
17. Heintz AP, Odicino F, Maisonneuve P, Quinn MA, Benedet JL, Creasman WT, et al. Carcinoma of the ovary. FIGO 26th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet 2006;95(Suppl 1):S161–92.
18. Duffy MJ, Bonfrer JM, Kulpa J, Rustin GJ, Soletormos G, Torre GC, et al. CA125 in ovarian cancer: European Group on Tumor Markers guidelines for clinical use. Int J Gynecol Cancer 2005;15:679–91.
19. Tuxen MK, Soletormos G, Dombernowsky P. Tumor markers in the management of patients with ovarian cancer. Cancer Treat Rev 1995;21:215–45.
20. Holcomb K, Vucetic Z, Miller MC, Knapp RC. Human epididymis protein 4 offers superior specificity in the differentiation of benign and malignant adnexal masses in premenopausal women. Am J Obstet Gynecol 2011;205:358e1–6.
21. Jacob F, Meier M, Caduff R, Goldstein D, Pochechueva T, Hacker N, et al. No benefit from combining HE4 and CA125 as ovarian tumor markers in a clinical setting. Gynecol Oncol 2011;121:487–91.
22. Liu YN, Ye X, Cheng HY, Cheng YX, Fu TY, Chen J, et al. [Measurement of serum human epididymis secretory protein 4 combined with CA125 assay in differential diagnosis of endometriosis cyst and ovarian benign and malignant tumors]. Zhonghua Fu Chan Ke Za Zhi 2010;45:363–6.