HE4 Tissue Expression and Serum HE4 Levels in Healthy Individuals and Patients with Benign or Malignant Tumors: A Systematic Review

Nikoline S. Karlsen[1], Mona A. Karlsen[1,2], Claus K. Høgdall[2], and Estrid V.S. Høgdall[1]

Abstract

Human epididymis protein 4 (HE4) has received major attention as a potential tumor marker in epithelial ovarian cancer; however, evidence of significant overexpression of HE4 in several other human cancers is expanding. To assess the possible limitations or benefits of HE4 in a clinical setting, this review aims to systematically outline published results of HE4 tissue expression and serum HE4 levels in healthy individuals and patients with benign or malignant tumors. Our findings suggest scientific basis for a potential diagnostic ability of HE4 in gynecologic cancer and lung cancer, and further research is needed regarding other cancers. Yet, it is important to recognize that other malignancies can cause increased HE4 levels. Furthermore, attention should be paid to the influence of age and renal function on HE4 serum levels in future studies as well as in the clinic for proper interpretation of serum HE4 test results. *Cancer Epidemiol Biomarkers Prev; 23(11); 2285–95. ©2014 AACR.*

Introduction

The rapid development in bioinformatics and molecular techniques has accelerated the intense search for tumor markers detectable in tissues and/or bodily fluids to correctly diagnose cancer or identifying subgroups of patients for special treatment. For this purpose, the glucoprotein human epididymis protein 4 (HE4) encoded by the Whey-Acidic Four-Disulfide Core domain protein 2 (WFDC2) gene has received attention (1). HE4 is a secretory protein originally identified in the distal human epididymis (2). The function of HE4 has not been definitely demonstrated; however, HE4 shows significant structural similarity to proteinase inhibitors and is proposed to have a function in sperm maturation (1–4). Widespread expression of HE4 has since been demonstrated in several normal tissues, especially in the epithelia of the respiratory and reproductive tracts of both genders except from the ovaries, where no expression is seen (5–8).

Increased HE4 tissue expression has been demonstrated in a range of malignant neoplasms, especially of gynecologic and pulmonary origin (5, 6, 9, 10). Corresponding, significantly elevated HE4 serum levels have been widely investigated in patients with ovarian cancer

(5, 7, 11–13), and large studies have reported serum HE4 as a putative tumor marker for differentiating between benign gynecologic tumors and ovarian cancer, claiming HE4 to be as good as the clinically approved serum cancer antigen 125 (CA125) used in ovarian cancer risk management worldwide (14–16). In 2008, the serum HE4 EIA analysis was cleared by the Food and Drug Administration (FDA) in the United States as a diagnostic tool to aid in the diagnosing process of ovarian cancer (17). So far, no comprehensive overview of HE4-expressing tissues and conditions influencing serum levels of HE4 exist. The aim of this review is to provide an outline of published studies investigating HE4 tissue expression in normal, benign, and malignant tissues, and to report the different clinical conditions in which serum HE4 levels are influenced. Possible limitations or benefits of HE4 in a clinical setting will be addressed.

Search Strategy and Selection Criteria

This systematic review was performed with guidance from the general principles of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. The PubMed and Scopus databases were used in search for literature. Two reviewers (N.S. Karlsen and M.A. Karlsen) were primarily responsible for literature search, and each reviewer double-checked the relevancy of the literature found. The keywords "Human Epididymis protein 4," "HE4," or "WFDC2" were combined with the terms "gene/tissue/protein expression," "serum/blood level/concentration," "bio/tumour marker," and "cancer/malignancies." When HE4-expressing tissues or conditions influencing serum HE4 concentration were revealed, the respective terms were used in further literature search (Fig. 1). We also performed a screening for

[1]Molecular Unit, Department of Pathology, Herlev University Hospital, Herlev, Denmark. [2]Gynecologic Department, The Juliane Marie Centre, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark.

Corresponding Author: Estrid V.S. Høgdall, Molecular Unit, Department of Pathology, Herlev University Hospital, Herlev Ringvej 75, 2730 Herlev, Denmark. Phone: 45-3868-9132; E-mail: estrid.hoegdall@regionh.dk

literature in reference sections of relevant studies. We aimed to cover a wide spectrum of published studies investigating RNA/protein expression and serum concen-tration of HE4, yet large and recent studies published in English were favored, whereas articles with no clear def-inition of methods to analyze expression or concentration
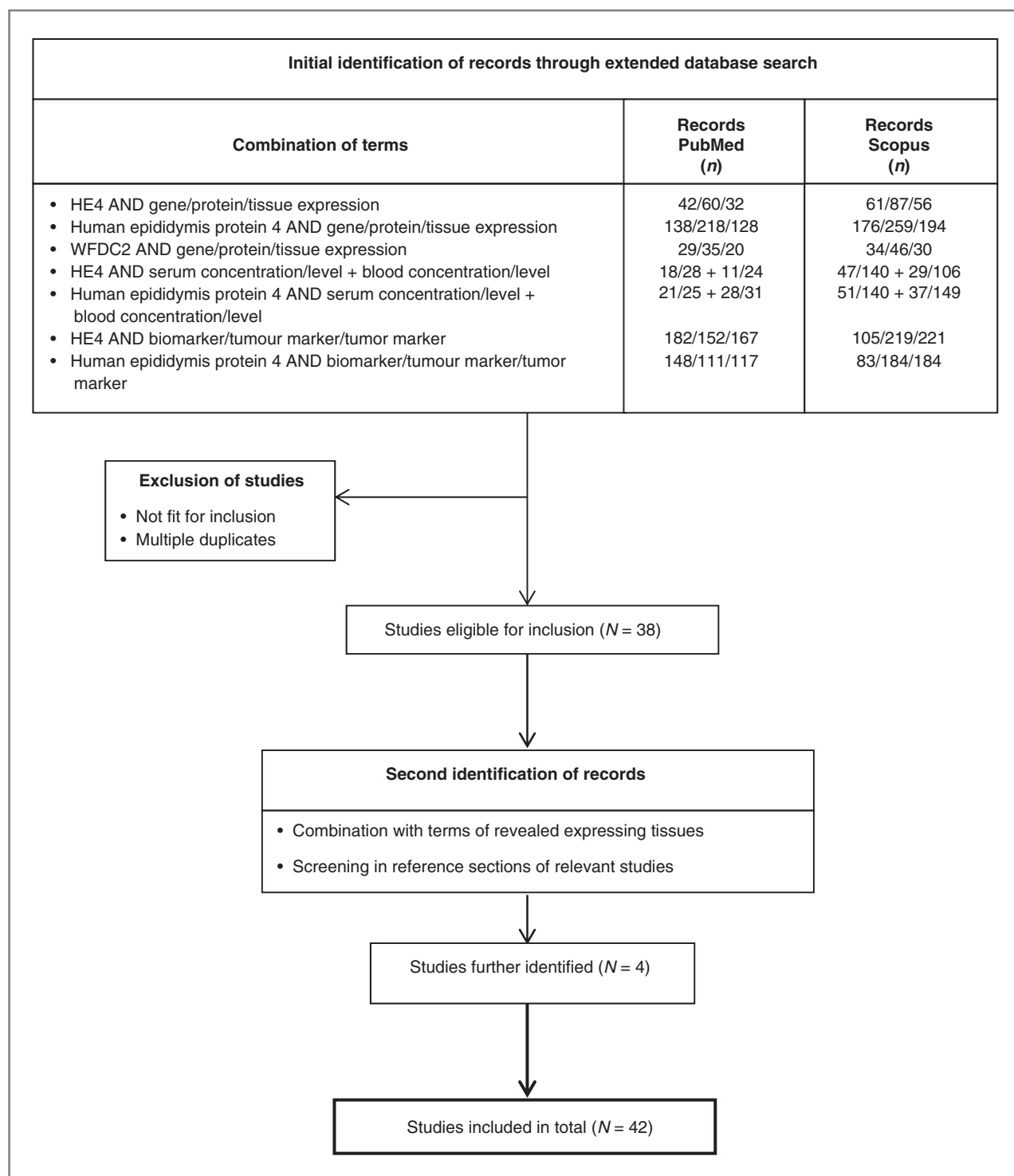
Initial identification of records through extended database search		
Combination of terms	**Records PubMed (*n*)**	**Records Scopus (*n*)**
• HE4 AND gene/protein/tissue expression	42/60/32	61/87/56
• Human epididymis protein 4 AND gene/protein/tissue expression	138/218/128	176/259/194
• WFDC2 AND gene/protein/tissue expression	29/35/20	34/46/30
• HE4 AND serum concentration/level + blood concentration/level	18/28 + 11/24	47/140 + 29/106
• Human epididymis protein 4 AND serum concentration/level + blood concentration/level	21/25 + 28/31	51/140 + 37/149
• HE4 AND biomarker/tumour marker/tumor marker	182/152/167	105/219/221
• Human epididymis protein 4 AND biomarker/tumour marker/tumor marker	148/111/117	83/184/184

Exclusion of studies

• Not fit for inclusion
• Multiple duplicates

Studies eligible for inclusion (*N* = 38)

Second identification of records

• Combination with terms of revealed expressing tissues

• Screening in reference sections of relevant studies

Studies further identified (*N* = 4)

Studies included in total (*N* = 42)

Figure 1. Flow diagram of the literature search and selection process in the PubMed and Scopus databases. Keywords: "Human epididymis protein 4," "HE4," or "WFDC2" were combined with the terms; "gene/tissue/protein expression," "serum/blood level/concentration," "bio/tumour marker," and "cancer/malignancies." When HE4-expressing tissues or conditions influencing serum HE4 concentration was revealed, the respective terms were used in further literature search. A total of 42 studies published from 2000 to 2014 were included.

levels in tissue or serum were excluded. A total of 42 studies published from 2000 to 2014 were included. Because of discrepancy in expression quantification among the studies included, a consistent definition of tissue expression levels was used in this review described as low, moderate, or high, based on the study results of the individual article as well as assessed in relation to other studies included in the review.

HE4 analyses

HE4 tissue expression and serum levels are analyzed by a variety of methods. HE4 RNA expression was for the majority of the referred studies measured as mRNA assessed by either the use of microarray technology or quantitative real-time PCR (qRT-PCR; Table 1). HE4 protein expression was measured by immunohistochemistry using either tissue microarray (TMA) or whole-tissue slides. Different HE4 antibodies were used in the reported studies (Table 1). High concordance has been demonstrated between the results obtained using microarray and qRT-PCR (>87%; $P < 0.05$; ref. 18); however, so far no study compared the concordance between protein expression levels using different HE4 antibodies. Serum HE4 is determined by HE4 immunoassays, and several standardized assays have been developed (19, 20). Different assays for measuring HE4 serum levels may not result in identical results. Discrepancy in HE4 levels has been demonstrated when comparing two different manufacturers' HE4 assays: Fujirebio HE4 assay measuring 14% (11.3%–16.9%) higher serum HE4 levels compared with the Abbott Architect assay (21). The influence of storage temperature on HE4 stability has only been investigated by one study, recommending HE4 serum and HE4 EDTA plasma to be stored at $-80°C$ for longer-term storage to maintain stability (22). For the majority of the studies included, serum samples have been stored at either $-80°C$ or $-70°C$ (Table 2).

Tables 1 and 2 demonstrate general study conditions, expression quantification, case numbers, and sensitivities and specificities of all studies included. In Table 1, it is further specified which studies had published microarray data of HE4 expression according to the Minimum Information About a Microarray Experiment (MIAME) guidelines (Table 1; refs. 23, 24).

Results

Normal tissues expressing HE4

The highest expression of HE4 RNA and protein are reported in the glandular epithelium of the female and male genital tracts and the respiratory tract (5, 6, 8). In the male genital tract, HE4 is highly expressed in the epithelial cells of the epididymal and spermatic ducts, whereas no expression is found in the surrounding stroma of the testes (5, 6). Low focal expression of HE4 is inconsistently found within the glandular epithelium of the prostate (5, 6) High HE4 expression in the female genital tract is seen in the fallopian tubes, endometrium, and cervical glands. Low HE4 expression is detected in the Bartholin glands, and no expression is seen in the myometrium, vulva, and ovaries (5, 6, 9). In the respiratory tract, HE4 is highly expressed in the epithelium of the oral cavity, the excretory ducts of the salivary glands, nasopharynx, and especially the trachea (5, 6, 8), whereas results of HE4 expression in the lungs are diverging. Three lung studies have detected HE4 RNA and protein expression in the lung tissue (5, 6, 8); however, two other studies could not confirm these findings (25, 26).

Focal and low HE4 expression is detected in the distal convoluted tubules of the kidney, and no expression is found in the rest of the kidneys, ureteres, bladder, and urethra (5, 6, 9). Breast epithelium demonstrated variably HE4 expression with higher staining in the ducts compared with the lobules (5, 6). Low and focal HE4 expression is seen in scattered cells in the anterior pituitary, thyroid, lacrimal, and eccrine sweat glands, whereas no expression is seen the adrenal cortex and medulla (5, 6). Expression is absent in the gastrointestinal canal (5, 6, 10, 27), as well as in hematolymphoid tissue (bone marrow, lymph nodes, spleen, thymus, and tonsils), musculoskeletal tissue (cartilage, fat, skeletal muscle, and synovium), neural tissue, skin, and vascular tissue (aorta, heart, and lymphatics; ref. 5).

HE4 expression in benign neoplasms

The amount of studies investigating HE4 tissue expression in benign tumors is sparse. To our knowledge, HE4 tissue expression has only been demonstrated in various benign ovarian tumors, endometriosis, preneoplastic metaplasias of the upper gastrointestinal canal, and oncocytomas of the kidney. One study investigated HE4 expression in benign ovarian tumors, including benign serous ($N = 12$) and mucinous ($N = 12$) cystadenomas, ovarian endometriosis ($N = 12$), and benign ovarian surface inclusion cysts ($N = 12$; ref. 9). Moderate-to-high HE4 tissue expression was detected in 11 of 12 mucinous cystadenomas and 11 of 12 ovarian surface inclusion cysts, whereas 5 of 11 serous cystadenomas demonstrated low expression. Moderate-to-high expression was detected in all samples of ovarian endometriosis analyzed (9). Furthermore, Drapkin and colleagues (6) found that HE4 expression in 11 ovarian surface inclusion cysts was mainly present in cysts lined by the Mullerian epithelium, compared with no HE4 expression in the cysts that kept the flat morphology of the ovarian surface epithelium. In the upper gastrointestinal canal, HE4 expression has been investigated in preneoplastic metaplasias of the esophagus, stomach, and pancreas. In 5 of 12 samples of Barret esophagus scattered HE4 expression was demonstrated (10). In the stomach, intestinal metaplasia and spasmolytic polypeptide–expressing metaplasia showed high HE4 expression (27). Furthermore, elevated HE4 tissue expression was demonstrated in gastric epithelium from stomachs infected with *Helicobacter pylori*, associated to gastric cancer (28). In the pancreas, pancreatic intraepithelial neoplasia has been investigated and no HE4 expression was seen in the tissues analyzed ($N = 55$; ref. 10). Finally,

Table 1. Study characteristics of included studies examining HE4 RNA and/or protein tissue expression

Reference	Country and year of study	Analyzing method		Control (+/−)	Expression quantification		Tissues investigated, n (RNA)			Tissues investigated, n (protein)		
		RNA	Protein (HE4 AB)		RNA	Protein	NT	BT	MT	NT	BT	MT
Galgano et al. (5)	USA 2006	OMA	TMA and WTS (poly-rAB)	+ HE	Relative intensity units: low (<300) moderate (300–999) high (≥1,000)	H-score: 0, negative 1–3, weak ≥4, strong	43		175	NI	12	436
Drapkin et al. (6)	USA 2005	RT-PCR	TMA (poly-rAB)	+ HE	NI	NI			14 CL	NI	11	NI
Bingle et al. (7)	England 2002	Northern blots of total RNA and multiple tissue Poly(A)+ RNA dot plot		NT	NI		50		6 CL			
Bingle et al. (8)	England 2006	RT-PCR	TMA (mono-mAB)	− r/mIgG	NI	Negative, focal, or positive				15		150
Georgakopoulos et al. (9)	USA 2012		WTS (NI)	+ HE	NI	Staining intensity (negative, weak, and strong) and proportion of positive cells			NI	36	72	56
O'Neal et al. (10)	USA 2013		TMA (rAB)	NI	NI	Scored on the basis of the median level as low or high				NI	67	>1,000
Yamashita et al. (25)	Japan 2011	RT-PCR	WTS (poly-rAB)	+ HE	NI	H-score: 0, negative 1–3, weak 4, strong	137		137	137		137
Iwahori et al. (26)	Japan 2012		WTS (poly-rAB)	NI	NI	NI				NI		NI
Nozaki et al. (27)	USA 2008	OMA and qPCR	TMA (rAB)	NI	NI	NI	NI	NI	NI	44	33	44
Hofman et al. (28)	France 2007	Semi-quantitative real-time PCR	TMA (poly-AB)	NT		Arbitrary units	15	42		69		
Bignotti et al. (29)	Italy 2011	Quantitative real-time PCR	TMA (poly-rAB)	NT	Relative quantification units	Score from 0 to 3	20		46	33		153
Huhtinen et al. (30)	Finland 2009	OMA		NI	NI		41	241	29			
Garber et al. (31)[a]	USA 2001	OMA		NI	Hierarchical clustering was used to interpret the patterns of expression		5		67			
Kamei et al. (32)	Japan 2010	RT-PCR	WTS (poly-rAB)	+ HE	NI	H-score: 0, negative 1–3, weak ≥4, strong			2 CL	129	33	129
Ryu et al. (33)	USA 2002	RT-PCR		NI	NI		2 CC		4 CL			

NOTE: + and − indicate positive and negative control, respectively.
Abbreviations: Mono-mAB, monoclonal mouse antibody; OMA, oligonucleotide microarray; poly-rAB, polyclonal rabbit antibody; WTS, whole-tissue slides.
[a]Describing and microarray data according to the MIAME guidelines.

Table 2. Study characteristics and SN and SP findings of included studies examining conditions influencing HE4 serum level

Reference	Country and year of study	Method and storage temperature	Cutoff, pmol/L	Cases in total (N)			SN and SP considered relevant are listed	
				Healthy	Benign tumor	Malignant tumor	SN	SP
Escudero et al. (13)	Spain/2011	A (−70°C)	140	101	292	400 various	NI	NI
Moore et al. (14)	USA/2008	F (−80°C)	NI		166	67 OC	All stage vs. benign: 72.9% Stage I vs. benign: 43.9%	95%[X] 95%[X]
Holcomb et al. (15)	USA/2011	F (NI)	70		195	18 OC	88.9%	91.8%
Karlsen et al. (16)	Denmark/2012	A (−80°C)	200 200 150^PostM 70^PreM		809	252 OC	OC vs. benign gyn.: 94.4%[X] 91.3% OC vs. all: 79.6% 81.8%	63.2% 75%[X] 82.5% 89.6%
Bolstad et al. (21)	Norway/2012	F and A (NI)	Age-depended cutoffs	1,591				
Yamashita et al. (25)	Japan/2011	F (−80°C)	32.2	37		40 LC	Cancer vs. healthy: 67.6%	88.3%
Iwahori et al. (26)	Japan/2012	F + E (−80°C)	6.25 ng/mL	37		85 various	LC vs. healthy: 89.8%	100%
Bignotti et al. (29)	Italy/2011	F (−80°C)		76^PostM		138 EC	67%	95%
Huhtinen et al. (30)	Finland/2009	F (−20°C/−80°C)	70	66	129	14 OC 16 EC	OC vs. endomet.: 71.4% OC vs. healthy: 78.6%	95% 95%
Urban et al. (34)	USA/2012	A (NI)	Age-depended cutoffs	1,780				
Hertlein et al. (35)	Germany/2012	A (−80 °C)	≤50 y ♀ = 96 ♂ = 120 >50 y ♀ = 81 ♂ = 93	204	654	704 various	OC vs. benign gyn.: 67.4% LC vs. benign lung: 11.1% ♀ 26.1% ♂ BC vs. benign breast: 18.0% ♀	95% 95% 95% 95%
Mokhtar et al. (36)	Malaysia/2012	A (−80°C)	69.0^PostM 58.4^PreM	300				
Nagy et al. (37)	Hungary/2014	A (−70°C)	97.6	98		98 LC	64.3%	95.9%

(Continued on the following page)

Table 2. Study characteristics and SN and SP findings of included studies examining conditions influencing HE4 serum level (Cont'd)

Reference	Country and year of study	Method and storage temperature	Cutoff, pmol/L	Cases in total (N)			SN and SP considered relevant are listed	
				Healthy	Benign tumor	Malignant tumor	SN	SP
Yang et al. (38)	China/2013	R (−80°C)	90.76^{PostM} 65.87^{PreM}	1,515				
Hallamaa et al. (39)	Finland/2012	F (−20°C/−80°C)	70	54	126		NI	NI
Anastasi et al. (40)	Italy/2010	F (−80°C)	150	40				
Nagy et al. (41)	Hungary/2012	A (NI)	140^{PostM} 70^{PreM}	181				
Park et al. (42)	Korea/2011	F (−70°C)	NI	16	85	60 various	OC vs. all: 44.8%	95%
Anastasi et al. (43)	Italy/2010	F (−80°C)	150	72	86	81 various	96% for OC	100%
Montagnana et al. (44)	Italy/2009	F (−80°C)	30	12	40	46 OC 53 EC + CC	OC vs. healthy: 98%	100%
Moore et al. (45)	USA/2012	F (NI)	128^{PostM} 89^{PreM} 114^{All}		1,042			
Havrilesky et al. (46)	USA/2008	E (−80°C)	1.8 ng/mL	500		200 OC	93.2%	73.7%
Moore et al. (47)	USA/2008	F (−80°C)	NI	156		171 EC	All stage vs. healthy: 45.5% Stage I vs. healthy: 37.9%	95%^{X} 95%^{X}
Antonsen et al. (48)	Denmark/2013	A (−80°C)	70		16	329 EC	43.9%	76.5%
Zanotti et al. (49)	Italy/2012	A (−80°C)	51	125		193 EC	78.8% 63.5%	84.8% 95%^{X}
Angioli et al. (50)	Italy/2012	F (−80°C)	70 150		103	101 EC	59.4% 35.6%	100% 100%
Ucar et al. (51)	Turkey/2014	F (−80°C)	67.5	19	38	64 LC	LC vs. all: 87.0%	60%
Liu et al. (52)	China/2013	F (−80°C)	94.01 77.48	106	138	190 LC	LC vs. benign lung: 61.6% LC vs. healthy: 67.9	93.0% 93.4
Xi et al. (54)	China/2009	F (−80°C)	45.7	60	60	102 bladder cancer	NI	NI
Elsammak et al. (53)	Saudi Arabia/2012	A (NI)	1.675 (pleural fluid)	54		Malign pleural effusion (34)	85.3%	90.7%

Abbreviations: A, CMIA Abbott ARCHITECT i2000SR System; BC, breast cancer; BOT, borderline ovarian tumors; CC, cervical cancer; CMIA, chemiluminescence microparticle immunoassay; E, 96-well ELISA plates; EC, endometrial cancer; Endomet, endometriosis; F, HE4 EIA Fujirebio Diagnostics; GI, gastrointestinal; Gyn, gynecologic; LC, lung cancer; NI, no information; OC, ovarian cancer; PostM, postmenopausal; PreM, premenopausal; R, ECLIA Roche Cobas E601 System; SN, sensitivity; SP, specificity; X, set specificities.

low to high expression was demonstrated in eight of 12 oncocytomas from the kidney (5).

HE4 expression in malignant neoplasms

In the female genital tract, high HE4 tissue expression is consistently found in epithelial ovarian adenocarcinomas of serous histology, whereas a lower and more inconsistent HE4 expression is found in endometrioid and clear cell histology. No HE4 expression is detected in epithelial ovarian cancer of mucinous histology (5, 6, 9), as well as no expression is present in nonepithelial ovarian cancer, including sex cord stromal tumors (nine granulosa cell tumors) and germ cell tumors (four dysgerminomas and a single yolk sac tumor; ref. 5). Furthermore, HE4 expression is reported in serous borderline tumors of the ovary, yet, with weaker expression compared with HE4 expression in epithelial ovarian cancer, and diverging results exist regarding the mucinous borderline ovarian tumors (5, 9). High HE4 expression has been demonstrated in primary tubal carcinomas ($N = 12$; ref. 9) and endometrial carcinomas (5, 6, 9, 29, 30). Bignotti and colleagues (29) found that HE4 tissue expression was significantly higher in endometrial carcinoma tissues compared with surrounding normal endometrium ($P < 0.0001$), and HE4 expression in the endometrioid subtype was significantly higher compared with nonendometrioid subtype ($P = 0.016$).

In the respiratory tract, one study has demonstrated moderate-to-high HE4 expression in various malignant neoplasms of the salivary gland ($N = 24$; ref. 5). In addition, HE4 expression has been demonstrated in several lung cancer cell lines (7, 31). Compared with small cell, squamous, and large cell lung cancer, adenocarcinomas of the lung demonstrate the highest HE4 tissue expression (5, 8, 25, 26). Finally, Galgano and colleagues (5) demonstrated low to high HE4 expression in 28 of 47 mesotheliomas investigated.

Two studies have investigated HE4 tissue expression in carcinomas of the breast, and found a moderate increase in HE4 expression of ductal carcinomas (5, 32).

In the gastrointestinal canal, HE4 tissue overexpression is seen in gastric cancer (27) and pancreatic cancer as well as occasionally in colon and hepatocellular cancer (5, 10, 33). In a study cohort comprising more than 550 tissue samples from all stages of gastric cancer, high HE4 tissue expression was demonstrated in 70% of intestinal type, in more than 90% of diffuse type and in 75% of mixed type (10). In 103 of 220 pancreatic ductal adenocarcinoma samples (172 individual patients), low HE4 expression was seen in more than 5% of the epithelial cancer cells (10), a finding supported by two other studies (5, 33). Low HE4 RNA expression was demonstrated in 18 of 23 colon cancer samples and all seven hepatocellular carcinomas investigated. However, when performing immunohistochemical staining for HE4 protein expression, merely 6 of 27 colon cancer samples demonstrated low HE4 protein expression, whereas no HE4 expression was detected in the hepatocellular carcinomas investigated (5).

In the urologic tract, low to high HE4 expression is seen in clear cell carcinomas of the kidney, papillary renal cell carcinomas, and chromophobe carcinomas of the kidney (5, 7, 11). Also transitional cell carcinomas have demonstrated high or moderate RNA expression in only two of eight samples investigated, whereas prostatic adenocarcinomas in the male genital tract show no expression of HE4 (5). Finally, Galgano and colleagues (5) demonstrated no HE4 expression in basal cell carcinomas of the skin.

Serum levels of HE4 in healthy individuals

In healthy individuals, serum HE4 significantly varies between sexes with a pronounced age-related increase (13, 21, 34–36). In a multivariate analysis with 1,591 samples comprising 801 women and 790 men of ages 18 to 86 years, age was shown to be the main determinant of serum HE4 level, and age-dependent reference limits were determined for men and women excluding all smokers. The lowest limit at the age of 18 years was 43.4 pmol/L for men and 51.3 pmol/L for women, whereas the highest limit at the age of 82 years was 78.4 pmol/L for men and 69.7 pmol/L for women (21). Significantly increased HE4 levels are also measured in smokers (29% increase) compared with nonsmokers ($P = 0.007$; refs. 21, 34, 37). Furthermore, significantly higher median HE4 levels in serum are measured in postmenopausal women compared with premenopausal ($P < 0.001$; refs. 36, 38), and serum HE4 varies within the different phases of the menstrual cycle (39, 40).

Finally, patients with renal failure have been demonstrated to have significantly increased serum HE4 concentration correlating to elevated serum creatinine levels (13, 21, 35, 41, 42). One study measured serum HE4 in a group of 113 female patients at different stages of chronic kidney disease with no history of ovarian cancer or lung cancer. The HE4 cutoff values were 70 pmol/L in premenopausal women and 140 pmol/L in postmenopausal women. Compared with 68 healthy females, a significant positive correlation between increasing severity of renal failure and elevated serum HE4 levels was found in both the pre- and postmenopausal group ($P = 0.003$–0.0001 and $P = 0.001$–0.0001, respectively; ref. 41). Liver disease has been proved to induce only small, yet, significant increases in serum HE4 levels ($P = 0.001$) in one study (13).

Serum levels of HE4 in benign and malignant neoplastic diseases

Significantly elevated levels of serum HE4 are detected in patients with epithelial ovarian cancer, endometrial cancer, lung adenocarcinomas, and transitional cell carcinomas when compared with serum HE4 levels in benign and healthy individuals. The most pronounced increase of serum HE4 is measured in patients with epithelial ovarian cancer, especially in serous and endometrioid epithelial ovarian cancer (13, 35). Using the serum HE4 concentration 70 pmol/L as cutoff value (14), serum HE4 has been demonstrated by several to be significantly increased in women with epithelial ovarian cancer compared with

healthy controls and various benign gynecologic diseases (13, 14, 16, 30, 42–44), where a modest serum HE4 increase can be detected (14, 30, 44, 45). HE4 has been demonstrated to yield a higher sensitivity and specificity than that of CA125 serum marker when differentiating epithelial ovarian cancer from benign gynecologic disease, and when serum HE4 is combined with serum CA125, the diagnostic accuracy increases even further (13–16, 42–44, 46). Serum HE4 is significantly elevated in women with endometrial cancer regardless of stage compared with healthy, postmenopausal women ($P < 0.001$; refs. 29, 30, 47, 48). For all stages of endometrial cancer ($N = 171$), Moore and colleagues (47) found that serum HE4 had a sensitivity of 45.5% at a predefined specificity of 95% compared with a sensitivity of 24.6% for CA125 when differentiating from healthy individuals, whereas Zanotti and colleagues (47) found a sensitivity at 66% for HE4 at a set specificity at 95%. Combined with CA125, the sensitivity increased to 50.1% (47). When distinguishing patients with endometrial cancer from women with benign disease of the uterus, Angioli and colleagues (50) found a sensitivity of 59.4% and a specificity of 100% at a HE4 cutoff level of 70 pmol/L.

Significantly elevated level of serum HE4 has been detected in patients with primary lung adenocarcinoma compared with levels in healthy control subjects and patients with benign lung tumor ($P = 0.0001$; refs. 13, 25, 26, 35, 37, 51, 52). One recent study investigated the diagnostic eligibility of serum HE4, and found a sensitivity of 67.9% at a specificity of 93.4% using an optimal cutoff value of 77.48 pmol/L when differentiating patients with lung cancer ($N = 190$) from healthy controls ($N = 106$; ref. 53). Nagy and colleagues (37, 52) found a sensitivity of 64.3% and specificity of 95.9% using a cutoff value of 97.6 pmol/L; however, HE4 was not shown to be superior to other tumor markers investigated: CEA, TPA, Cyfra 21-1, and CA125. Only if serum HE4 was combined with CEA and CA125, the diagnostic efficacy was enhanced yielding a sensitivity of 91.8% and a specificity of 92.8% (37).

In a study comprising 222 serum samples, HE4 serum levels were significantly higher in patients with transitional cell carcinomas ($N = 102$; median, 66.7 pmol/L; mean, 42.1 ± 108.8), compared with HE4 levels in patients with benign urinary diseases ($N = 60$; median, 58.3 pmol/L; range, 51.8–63.6; $P < 0.001$) and healthy controls ($N = 60$; median, 37.3 pmol/L; range, 28.9–44.4; $P < 0.01$). The sensitivity and specificity of HE4 at a cutoff value of 45.7 pmol/L were 67.6% and 88.3%, respectively (54).

Park and colleagues (42) found that serum HE4 level was significantly increased in patients with pancreatic cancer measuring a median of 213 pmol/L. Yet three other studies investigating serum HE4 levels in patients with various malignancies of the gastrointestinal canal, including pancreatic cancer, found only a nonsignificant elevation compared with levels found in healthy patients (13, 26, 43).

Escudero and colleagues (13) found that serum HE4 was significantly related to the presence of liver metastases from any cancer origin ($P = 0.001$). Furthermore, Elsammak and colleagues found that in 88 patients with different types of pleural effusions, both serum HE4 levels and pleural effusion HE4 levels were significantly higher in patients with malignant effusions than in patients with transudative or nonmalignant exudative effusions ($P < 0.001$ and $P = 0.002$, respectively). In pleural fluid a cutoff value of 1.675 pmol/L was found to predict malignant pleural effusions with a diagnostic sensitivity of 85.3% and specificity of 90.7% (53).

Discussion

The purpose of this review was to provide a comprehensive outline of HE4 expression in normal, benign, and malignant human tissues and HE4 serum levels to evaluate the clinical eligibility of HE4 as a potential tumor marker. The vision of HE4 as a potential tumor marker has expanded through the past decades due to an increase of studies demonstrating an upregulation of HE4 in various meta- and neoplasias occasionally followed by elevated levels of the substance in blood.

A prominent upregulation of HE4 expression was seen in epithelial ovarian cancer tissue, especially in serous and endometrioid adenocarnomas. No expression was detected in normal ovarian tissue, and a lower expression was observed in both benign and borderline ovarian tumors compared with protein expression levels in epithelial ovarian cancer. This finding is clinically relevant, because the morphologic distribution of HE4 protein expression in whole-tissue slides or TMA's could be a marker used in the diagnostic process of epithelial ovarian cancer. Furthermore, epithelial ovarian cancer can be differentiated from nonepithelial ovarian cancer, because no expression was demonstrated in nonepithelial ovarian cancer. A significantly higher level of HE4 in serum was also detected in women with epithelial ovarian cancer compared with the HE4 serum levels in healthy women and women with a benign disease. In combination with serum marker CA125, a significant increase in sensitivity and specificity was revealed in differentiating between benign gynecologic conditions and epithelial ovarian cancer. Differentiation between benign and malignant diseases is of great importance for correct referral of patients to a tertiary center and thereby optimal treatment and follow-up (55–59). When investigating tissue from the fallopian tube and endometrium, a positive HE4 expression should be more carefully interpreted because a high HE4 expression was present in normal tissue. However, we cannot decline the potential of a HE4 as a tumor marker in endometrial cancer, because HE4 measured in serum from patients with endometrial cancer was significantly elevated compared with serum levels in healthy individuals and women with benign uterine disease, yet, further investigation is needed. Furthermore, additional investigation of HE4 expression pattern in tissue from cervical, vaginal, and

vulva cancer from tissue is needed to fully understand the relevance of HE4 in these cancers.

HE4 could potentially be a tumor marker in primary lung adenocarcinomas. A significant upregulation of HE4 was observed in lung cancer tissue compared with normal lung tissue and other primary lung cancers, where none or low expression was present. A significant elevated HE4 level in both serum and pleural fluid was detected in patients with primary lung adenocarcinomas compared with HE4 levels healthy control subjects and in patients with a benign lung tumor. However, to apply HE4 as a diagnostic tumor marker in a clinical setting, further clarification about tissue expression and serum levels in benign versus malignant lung disease as well as substudies of different lung cancers is needed.

Upregulation of HE4 was also demonstrated in malignancies of the gastrointestinal canal, urinary tract, bladder, and breast. However, these findings were demonstrated in few studies investigating relatively small tissue and serum materials. Therefore, we cannot make any conclusion about the clinical eligibility of HE4 as a tissue and serum tumor marker in these cancers, because larger studies are necessary before any statement can be taken. In addition, the inevitable heterogeneity in study conditions regarding specimen storage and analytic methods (Table 1 and 2) could have an impact on study results and also the influence of age, menstrual status, smoking habit, and renal function has on serum HE4 levels in healthy subjects were not considered in many study cohorts.

The widespread upregulation of HE4 seen in a range of malignancies implies that HE4 is neither organ nor tumor specific. Therefore, clinicians must be aware of the possibility for differential diagnoses in case of upregulated HE4 tissue expression or elevated levels of HE4 in serum. The implementation of the HE4 tumor marker test in the clinic would first of all require standardized preanalytic conditions, including specimen handling, storage, and analyzing method (60). Second, a set of well-estimated baseline concentrations should be prepared. So far, no optimal cutoff exist, as well as there is no consensus for correct parameters to include in the examination. Bolstad

and colleagues (21) have suggested a set of baseline serum HE4 concentrations for men and women adjusted for age, and two recent studies (36, 38) have suggested reference intervals of serum HE4 in healthy women at 65.87 pmol/L for premenopausal and 90.76 pmol/L for postmenopausal measured in the study with the largest group included ($N = 1,515$ healthy women; ref. 38). In women with pelvic masses, Fujirebio Diagnostics (19) has defined the normal range below 150 pmol/L, whereas Abbott Diagnostics (20) defined normal ranges below 70 pmol/L for premenopausal women and 140 pmol/L for postmenopausal women. However, both the manufacturers recommend that reference intervals are determined for each population investigated yielding the highest sensitivity and specificity possible.

Conclusion

There is a valuable diagnostic eligibility of HE4 tissue expression and serum levels in patients with a risk of gynecologic malignancies, especially epithelial ovarian cancer. The diagnostic ability of HE4 in patients with risk of lung adenocarcinomas seems promising. More research is needed to correctly evaluate the diagnostic relevance of HE4 in other cancers; however, it is important to recognize that other malignancies can cause increased HE4 expression and HE4 serum levels. Finally, an awareness of the influence of age, menstrual status, smoking habits, and renal function may have on serum level is important for proper interpretation of a serum HE4 test result.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Clauss A, Lilja H, Lundwall A. A locus on human chromosome 20 contains several genes expressing protease inhibitor domains with homology to whey acidic protein. Biochem J 2002;368:233–42.
2. Kirchhoff C, Habben I, Ivell R, Krull N. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. Biol Reprod 1991;45:350–7.
3. Kirchhoff C. Molecular characterization of epididymal proteins. Rev Reprod 1998;3:86–95.
4. 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.
5. Galgano MT, Hampton GM, Frierson HF Jr. Comprehensive analysis of HE4 expression in normal and malignant human tissues. Mod Pathol 2006;19:847–53.
6. 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.
7. Bingle L, Singleton V, Bingle CD. The putative ovarian tumour marker gene HE4 (WFDC2), is expressed in normal tissues and undergoes complex alternative splicing to yield multiple protein isoforms. Oncogene 2002;21:2768–73.
8. Bingle L, Cross SS, High AS, Wallace WA, Rassl D, Yuan G, et al. WFDC2 (HE4): a potential role in the innate immunity of the oral cavity and respiratory tract and the development of adenocarcinomas of the lung. Respir Res 2006;7:61.
9. Georgakopoulos P, Mehmood S, Akalin A, Shroyer KR. Immunohistochemical localization of HE4 in benign, borderline, and malignant lesions of the ovary. Int J Gynecol Pathol 2012;31:517–23.
10. O'Neal RL, Nam KT, Lafleur BJ, Barlow B, Nozaki K, Lee HJ, et al. Human epididymis protein 4 is up-regulated in gastric and pancreatic adenocarcinomas. Hum Pathol 2013;44:734–42.

11. Ross DT, Scherf U, Eisen MB, Perou CM, Rees C, Spellman P, et al. Systematic variation in gene expression patterns in human cancer cell lines. Nat Genet 2000;24:227–35.

12. Bouchard D, Morisset D, Bourbonnais Y, Tremblay GM. Proteins with whey-acidic-protein motifs and cancer. Lancet Oncol 2006;7:167–74.

13. 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.

14. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol 2008;108:402–8.

15. 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;205:358–6.

16. Karlsen MA, Sandhu N, Hogdall C, Christensen IJ, Nedergaard L, Lundvall L, et al. Evaluation of HE4, CA125, risk of ovarian malignancy algorithm (ROMA) and risk of malignancy index (RMI) as diagnostic tools of epithelial ovarian cancer in patients with a pelvic mass. Gynecol Oncol 2012;127:379–83.

17. FDA [homepage on the Internet]. U.S. Food and Drug Administration. [updated 2014 Aug 11; cited 2014 Aug 12]. Available from: http://fda.gov.

18. Dallas PB, Gottardo NG, Firth MJ, Beesley AH, Hoffmann K, Terry PA, et al. Gene expression levels assessed by oligonucleotide microarray analysis and quantitative real-time RT-PCR—how well do they correlate? BMC Genomics 2005;6:59.

19. Fujirebio Diagnostics [homepage on the Internet]. HE4 EIA—Instructions for Use [24 pages]; c2008–06 [cited 2014 Aug 12]. Available from: http://www.fdi.com/documents/products/inserts/eia/HE4%20EIA%20404-10US%202008-06%20%20F5908%20r1%20r0%20(3).pdf.

20. Abbott Diagnostics [homepage on the Internet]. HE4 and CA125 Information [about 4 screens]. [updated 2014; cited 2014 Aug 12]. Available from: https://www.abbottdiagnostics.com/en-int/HE4-CA125.

21. Bolstad N, Oijordsbakken M, Nustad K, Bjerner J. Human epididymis protein 4 reference limits and natural variation in a Nordic reference population. Tumour Biol 2012;33:141–8.

22. Sandhu N, Karlsen MA, Hogdall C, Laursen IA, Christensen IJ, Hogdall EV. Stability of HE4 and CA125 in blood samples from patients diagnosed with ovarian cancer. Scand J Clin Lab Invest. 2014 Apr 13. [Epub ahead of print].

23. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spellman P, Stoeckert C, et al. Minimum information about a microarray experiment (MIAME)-toward standards for microarray data. Nat Genet 2001;29:365–71.

24. Minimum Information About a Microarray Experiment—MIAME FGED Society; 2010. (Cited 2014 Aug 12.) Available from: http://www.mged.org/Workgroups/MIAME/miame.html.

25. Yamashita S, Tokuishi K, Hashimoto T, Moroga T, Kamei M, Ono K, et al. Prognostic significance of HE4 expression in pulmonary adenocarcinoma. Tumour Biol 2011;32:265–71.

26. Iwahori K, Suzuki H, Kishi Y, Fujii Y, Uehara R, Okamoto N, et al. Serum HE4 as a diagnostic and prognostic marker for lung cancer. Tumour Biol 2012;33:1141–9.

27. Nozaki K, Ogawa M, Williams JA, Lafleur BJ, Ng V, Drapkin RI, et al. A molecular signature of gastric metaplasia arising in response to acute parietal cell loss. Gastroenterology 2008;134:511–22.

28. Hofman VJ, Moreilhon C, Brest PD, Lassalle S, Le BK, Sicard D, et al. Gene expression profiling in human gastric mucosa infected with Helicobacter pylori. Mod Pathol 2007;20:974–89.

29. Bignotti E, Ragnoli M, Zanotti L, Calza S, Falchetti M, Lonardi S, et al. Diagnostic and prognostic impact of serum HE4 detection in endometrial carcinoma patients. Br J Cancer 2011;104:1418–25.

30. 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.

31. Garber ME, Troyanskaya OG, Schluens K, Petersen S, Thaesler Z, Pacyna-Gengelbach M, et al. Diversity of gene expression in adenocarcinoma of the lung. Proc Natl Acad Sci U S A 2001;98:13784–9.

32. Kamei M, Yamashita S, Tokuishi K, Hashioto T, Moroga T, Suehiro S, et al. HE4 expression can be associated with lymph node metastases and disease-free survival in breast cancer. Anticancer Res 2010;30:4779–83.

33. Ryu B, Jones J, Blades NJ, Parmigiani G, Hollingsworth MA, Hruban RH, et al. Relationships and differentially expressed genes among pancreatic cancers examined by large-scale serial analysis of gene expression. Cancer Res 2002;62:819–26.

34. Urban N, Thorpe J, Karlan BY, McIntosh MW, Palomares MR, Daly MB, et al. Interpretation of single and serial measures of HE4 and CA125 in asymptomatic women at high risk for ovarian cancer. Cancer Epidemiol Biomarkers Prev 2012;21:2087–94.

35. Hertlein L, Stieber P, Kirschenhofer A, Krocker K, Nagel D, Lenhard M, et al. Human epididymis protein 4 (HE4) in benign and malignant diseases. Clin Chem Lab Med 2012;50:2181–8.

36. Mokhtar N, Thevarajah M, Ma N, M I. Human epididymis protein 4 reference intervals in a multiethnic asian women population. Asian Pac J Cancer Prev 2012;13:6391–5.

37. Nagy B, Bhattoa HP, Steiber Z, Csoban M, Szilasi M, Mehes G, et al. Serum human epididymis protein 4 (HE4) as a tumor marker in men with lung cancer. Clin Chem Lab Med. 2014 May 15. [Epub ahead of print].

38. Yang J, Sa M, Huang M, Yang J, Xiang Z, Liu B, et al. The reference intervals for HE4, CA125 and ROMA in healthy female with electro-chemiluminescence immunoassay. Clin Biochem 2013;46:1705–8.

39. Hallamaa M, Suvitie P, Huhtinen K, Matomaki J, Poutanen M, Perheentupa A. Serum HE4 concentration is not dependent on menstrual cycle or hormonal treatment among endometriosis patients and healthy premenopausal women. Gynecol Oncol 2012;125:667–72.

40. Anastasi E, Granato T, Marchei GG, Viggiani V, Colaprisca B, Comploj S, et al. Ovarian tumor marker HE4 is differently expressed during the phases of the menstrual cycle in healthy young women. Tumour Biol 2010;31:411–5.

41. Nagy B Jr, Krasznai ZT, Balla H, Csoban M, Antal-Szalmas P, Hernadi Z, et al. Elevated human epididymis protein 4 concentrations in chronic kidney disease. Ann Clin Biochem 2012;49:377–80.

42. 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.

43. Anastasi E, Marchei GG, Viggiani V, Gennarini G, Frati L, Reale MG. HE4: new potential early biomarker for the recurrence of ovarian cancer. Tumour Biol 2010;31:113–9.

44. 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.

45. Moore RG, Miller MC, Steinhoff MM, Skates SJ, Lu KH, Lambert-Messerlian G, et al. Serum HE4 levels are less frequently elevated than CA125 in women with benign gynecologic disorders. Am J Obstet Gynecol 2012;206:351–8.

46. Havrilesky LJ, Whitehead CM, Rubatt JM, Cheek RL, Groelke J, He Q, et al. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. Gynecol Oncol 2008;110:374–82.

47. Moore RG, Brown AK, Miller MC, Badgwell D, Lu Z, Allard WJ, et al. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. Gynecol Oncol 2008;110:196–201.

48. Antonsen SL, Hogdall E, Christensen IJ, Lydolph M, Tabor A, Loft JA, et al. HE4 and CA125 levels in the preoperative assessment of endometrial cancer patients: a prospective multicenter study (ENDO-MET). Acta Obstet Gynecol Scand 2013;92:1313–22.

49. Zanotti L, Bignotti E, Calza S, Bandiera E, Ruggeri G, Galli C, et al. Human epididymis protein 4 as a serum marker for diagnosis of endometrial carcinoma and prediction of clinical outcome. Clin Chem Lab Med 2012;50:2189–98.

50. Angioli R, Plotti F, Capriglione S, Montera R, Damiani P, Ricciardi R, et al. The role of novel biomarker HE4 in endometrial cancer: a case control prospective study. Tumour Biol 2013;34:571–6.

51. Ucar EY, Ozkaya AL, Araz O, Akgun M, Meral M, Kaynar H, et al. Serum and bronchial aspiration fluid HE-4 levels in lung cancer. Tumour Biol. 2014 May 31. [Epub ahead of print].

52. Liu W, Yang J, Chi PD, Zheng X, Dai SQ, Chen H, et al. Evaluating the clinical significance of serum HE4 levels in lung cancer and pulmonary tuberculosis. Int J Tuberc Lung Dis 2013;17:1346–53.

53. Elsammak MY, Attia A, Hassan HA, Zaytoun TM, Shorman M, Suleman M. Evaluation of pleural fluid human epididymis 4 (HE4) as a marker of malignant pleural effusion. Tumour Biol 2012;33:1701–7.

54. Xi Z, LinLin M, Ye T. Human epididymis protein 4 is a biomarker for transitional cell carcinoma in the urinary system. J Clin Lab Anal 2009;23:357–61.

55. Earle CC, Schrag D, Neville BA, Yabroff KR, Topor M, Fahey A, et al. Effect of surgeon specialty on processes of care and outcomes for ovarian cancer patients. J Natl Cancer Inst 2006;98: 172–80.

56. Giede KC, Kieser K, Dodge J, Rosen B. Who should operate on patients with ovarian cancer? An evidence-based review. Gynecol Oncol 2005;99:447–61.

57. Kumpulainen S, Kuoppala T, Leminen A, Penttinen J, Puistola U, Pukkala E, et al. Surgical treatment of ovarian cancer in different hospital categories—a prospective nation-wide study in Finland. Eur J Cancer 2006;42:388–95.

58. Paulsen T, Kjaerheim K, Kaern J, Tretli S, Trope C. Improved short-term survival for advanced ovarian, tubal, and peritoneal cancer patients operated at teaching hospitals. Int J Gynecol Cancer 2006;16 (suppl 1):11–7.

59. Fago-Olsen CL, Hogdall C, Kehlet H, Christensen IJ, Ottesen B. Centralized treatment of advanced stages of ovarian cancer improves survival: a nationwide Danish survey. Acta Obstet Gynecol Scand 2011;90:273–9.

60. Sturgeon CM, Hoffman BR, Chan DW, Ch'ng SL, Hammond E, Hayes DF, et al. National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines for use of tumor markers in clinical practice: quality requirements. Clin Chem 2008;54:e1–10.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

HE4 Tissue Expression and Serum HE4 Levels in Healthy Individuals and Patients with Benign or Malignant Tumors: A Systematic Review

Nikoline S. Karlsen, Mona A. Karlsen, Claus K. Høgdall, et al.

Updated version	Access the most recent version of this article at: doi:10.1158/1055-9965.EPI-14-0447

Cited articles	This article cites 53 articles, 12 of which you can access for free at: http://cebp.aacrjournals.org/content/23/11/2285.full.html#ref-list-1

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.