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Serum human epididymis protein 4 (HE4) as a tumor marker in men with lung cancer

Abstract

Background: Human epididymis protein 4 (HE4) is a reliable tumor marker for ovarian cancer, but only limited data are available on HE4 levels in lung malignancies.

Methods: HE4 levels were measured at diagnosis in 98 men with lung cancer at different stages of the disease, and these results were compared to an age-matched healthy male cohort (n=98). The concentrations of classical tumor markers were also determined, and their efficacy was compared to that of HE4.

Results: Compared to healthy controls, patients with lung neoplasm showed significantly higher HE4 levels [118.2 (80.6–150.1) pmol/L vs. 62.2 (47.2–76.1) pmol/L; p<0.001]. Although age and smoking modulated HE4 levels in the healthy cohort, no such effect was observed in the patient population. The area under the receiver operating characteristic curve (ROC-AUC) for HE4 was 0.848 (95% CI 0.792–0.904) for differentiating lung cancer patients from healthy controls, with a cut-off value of 97.6 pmol/L (sensitivity: 64.3%, specificity: 95.9%). HE4 levels were significantly elevated in all stages of lung cancer, and even in patients without clinical symptoms (p<0.05), but no difference was found between the different histological subgroups. A significant correlation was found between HE4 values and the tumor size determined by CT/MRI (Spearman's ρ=0.227, p=0.030). The combination of HE4 with CEA and CA 125 considerably enhanced the diagnostic efficacy [ROC-AUC: 0.963 (95% CI 0.937–0.990), sensitivity: 91.8%, specificity: 92.8%].

Conclusions: Our data suggest that serum HE4, especially in combination with CEA and CA 125, qualifies as a surrogate diagnostic marker in men with lung cancer.

Keywords: HE4; lung cancer; non-small cell lung cancer (NSCLC); small cell lung cancer (SCLC); tumor marker.

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Introduction

Lung cancer is the most common malignancy in men, representing 16.5% of all cancer cases, and is the leading cause of tumor-related mortality in males [1]. Non-small cell lung cancer (NSCLC) accounts for about 85%, and small cell lung cancer (SCLC) represents the remainder of this malignancy [2, 3]. Epidemiological surveys report a 5-year survival rate of 15%; this poor prognosis is in part due to the delayed diagnosis, usually at a later stage of the disease [1–3]. To date, radiological screening for lung cancer is not recommended [4], and currently available tumor markers show rather variable sensitivity and specificity in lung tumors, especially in the early stages of the disease [5, 6]. As such, novel biomarkers with favorable diagnostic and prognostic value are desirable.

Human epididymis protein 4 (HE4) is a product of the whey-acidic-protein 4-disulphide core domain 2 (WFDC2) gene overexpressed in several types of cancer [7–9]. Compared to cancer antigen 125 (CA 125), HE4 is reported as having superior potential in the detection and progression of ovarian cancer in quite a few clinical studies [8, 10–13], i.e., HE4 showed high sensitivity and specificity in premenopausal and postmenopausal women [13]. Additionally, a negative predictive value of HE4 together with CA 125 was effective in the discrimination of benign and malignant gynecological diseases [14–16].

Abnormal HE4 immunoreactivity was first detected in tissue microarrays from pulmonary adenocarcinoma and

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squamous cell carcinoma [9]. This finding is attributed to the overexpression of WFDC2 gene in lung neoplasms [17]. Different splice variants of HE4 (V1-4) at moderate or high level were shown in lung adenocarcinoma cell lines [7], in accordance with a latest study demonstrating a 92.1% positivity of the HE4-V3 variant [18]. Thus, elevated serum HE4 levels are anticipated in this malignancy, but only limited data are available in this regards [12, 19–21].

In this prospective, case-control study, we measured serum concentrations of HE4 in de novo samples obtained at diagnosis of lung cancer in men who were still therapy naive, and in an equal number of age-matched healthy men. In order to avoid any potential effects of sex on HE4 levels, such as the presence of pelvic disorders [10, 13] and the effect of menstrual cycle [22], only men were included in this study. The levels of routine serum tumor markers for lung cancer were also measured, and their sensitivities and specificities were compared to that of HE4. We evaluated the effect of different confounders, and the relationship between clinical and histopathological data and HE4 level. Finally, binary logistic regression was used to model the combination of HE4 with different classic tumor markers in discriminating cancer patients from healthy controls.

Materials and methods

Study populations

Men (n=98) presenting with de novo lung cancer between March 2010 and April 2012 were included in this cross-sectional, analyst-blinded, age- and sex-matched case-control study. The median age (range) of the individuals in both groups was 62 (40–80) years, and 50% (n=49) of the cancer patients and 12% (n=12) of the controls were present smokers. During the recruitment period, cancer-suspect patients with variable pulmonary symptoms were admitted to the Department of Pulmonology, where detailed physical, radiological and laboratory examinations were performed before initiation of tumor-specific treatment. Serum samples for HE4 and other tumor marker [CA 125, Cyfra 21-1, carcinoembryonic antigen (CEA), tissue polypeptide antigen (TPA), thymidine kinase (TK), neuron-specific enolase (NSE)] determinations were obtained at the baseline visit. Tissue biopsy results revealed that 69 patients had NSCLC (33: squamous cell carcinoma; 31: adenocarcinoma; 5: large cell carcinoma), 15 patients were shown to have SCLC and no histological data were available in 14 cases. In the absence of histological data, diagnosis of lung cancer was based on CT or MRI findings. As per clinical staging, 13 were in stage I, eight were in stage II, 38 were in stage III and 39 were in stage IV of the disease based on the 7th TNM classification of lung cancers [23]. The age-matched healthy men were recruited from blood donors and staff of the faculty with values ≤2× upper limit of normal for all measured laboratory parameters. Exclusion criteria were cancer, metabolic and inflammatory diseases.

Ethics statement

This study was approved by the Regional Ethics Committee of the University of Debrecen (permit number: 3140-2010) in accordance with the Declaration of Helsinki. All participants gave written informed consents.

Laboratory methods

Blood samples were obtained by venous puncture, then centrifuged, and stored at –70 °C until analysis. Chemiluminescent microparticle immunoassay (Architect i2000SR®, ABBOTT) was used for analyzing serum concentrations of HE4, while an electrochemiluminescent immunoassay (Cobas e602®, Roche Diagnostics) was used to determine the levels of CEA, NSE, CA 125, and Cyfra 21-1. TPA and TK levels were determined by a chemiluminescent immunoassay (Liason®, DiaSorin). Renal function was evaluated by measuring serum creatinine levels (Cobas c702®, Roche), and estimated glomerular filtration rate (eGFR) (mL/min/1.73 m^2) was calculated using the 4v-MDRD (Modification of Diet in Renal Disease) formula [24].

Statistical analyses

Kolmogorov-Smirnov test was used for the evaluation of the normality of the data. Most parameters were non-normally distributed; therefore analyses were performed by Mann-Whitney U-test. The χ^2-test was used to compare categorical variables. The Spearman's ρ was calculated for correlation analysis. The univariate analysis of variance was used to adjust for independent factors. Paired-sample test was used to compare the age-matched groups. The prognostic effect of HE4 was evaluated to death or last follow-up by using Kaplan-Meier method and Log rank and Breslow tests. $p<0.05$ was regarded as statistically significant. Binary logistic regression was used to model the combination of HE4 with different standard tumor markers in discriminating cancer patients from healthy controls and the discriminative power of individual and combined markers was evaluated by receiver operating characteristics (ROC) curve analysis. All analyses were performed using the SPSS Statistics software, version 19.0 (IBM Corps., Armonk, NY, USA).

Results

HE4 and its confounders in patients and controls

There was no significant difference in serum creatinine concentrations (mean±SD: 70±14 μmol/L vs. 80±13 μmol/L), or eGFR (104±26 mL/min/1.73 m^2 vs. 88±18 mL/min/1.73 m^2) between the age-matched groups. As compared to their healthy counterparts, the levels of the classical tumor markers and HE4 were significantly higher ($p<0.001$) in the cancer group (Table 1).

Table 1 Serum tumor markers in healthy controls and lung cancer patients.

	HE4, pmol/L	CA 125, kIU/L	CEA, µg/L	Cyfra 21-1, µg/L	NSE, µg/L	TPA, U/L	TK, U/L
Healthy subjects	62.2 (47.2–76.1)[a]	9.8 (6.8–12.9)	1.9 (1.4–2.5)	1.4 (1.2–2.1)	6.3 (4.0–9.6)	43.2 (31.8–63.3)	7.6 (5.9–10.8)
All lung cancer patients	118.2 (80.6–150.1)[d]	40.5 (17.0–78.5)[d]	5.3 (3.2–15.0)[d]	4.5 (2.3–9.7)[d]	16.5 (4.5–34.8)[d]	114.0 (70.0–246.0)[d]	10.1 (6.3–19.4)[d]
Stage I	70.6 (60.5–114.4)[b]	15.6 (12.5–18.7)[d]	3.4 (3.2–5.7)[d]	1.6 (1.2–3.1)	4.6 (3.4–7.4)	35.3 (26.9–90.9)	4.5 (2.7–7.9)
Stage II	133.9 (65.2–141.5)[d]	12.3 (7.3–51.4)[b]	4.8 (2.7–9.1)[d]	2.5 (1.7–3.9)[b]	4.1 (2.9–8.1)	74.1 (32.5–98.6)	7.8 (5.5–12.5)[b]
Stage III	118.7 (79.9–153.1)[c]	49.9 (17.9–84.2)[d]	5.4 (3.4–13.2)[d]	6.1 (2.5–10.0)[d]	6.6 (4.7–10.3)[b]	129.0 (75.1–246.3)[d]	9.9 (6.3–17.2)[b]
Stage IV	135.9 (97.8–193.7)[d]	62.1 (25.4–108.0)[d]	14.6 (3.4–28.4)[d]	6.2 (3.4–16.3)[d]	7.3 (4.3–11.9)[b]	147.0 (90.5–393.0)[d]	14.8 (7.5–42.4)[d]
Squamous cell cc.	120.5 (68.9–143.7)[d]	48.5 (15.2–98.0)[d]	4.0 (2.8–10.4)[d]	5.3 (2.1–8.7)[d]	5.1 (3.6–8.6)[b]	111.0 (61.2–174.5)[d]	8.3 (5.9–15.2)
Adenocarcinoma	97.8 (70.6–142.6)[d]	18.4 (14.6–68.2)[d]	7.8 (3.8–19.4)[d]	3.8 (2.1–10.8)[d]	6.4 (4.3–9.2)[b]	117.0 (59.2–263.0)[d]	9.6 (7.0–17.0)[b]
Large cell cc.	192.9 (128.8–408.4)[d]	76.0 (51.3–148.0)[d]	6.8 (4.2–18.1)[d]	6.2 (2.2–19.0)[c]	4.7 (3.9–9.2)	115.0 (65.3–395.5)[c]	14.3 (6.7–28.4)[b]
Small cell cc.	120.7 (108.3–233.6)[d]	35.2 (24.7–63.9)[d]	6.9 (3.5–15.4)[d]	3.7 (2.5–9.7)[d]	27.4 (5.9–94.3)[d]	128.0 (79.8–352.0)[d]	56.2 (13.1–77.3)[d]

[a]Median value (range); [b]p<0.05; [c]p<0.01; [d]p<0.001 compared to healthy subjects.

The confounders of HE4 levels were analyzed in both study groups. Statistically significant positive correlation between HE4 levels and age (Spearman's ρ=0.374, p<0.001) was found only in the healthy men. Using the median age of 62 years, the study populations were divided into a middle-aged (≤62 years) and an elderly (>62 years) subgroup. Compared to healthy middle-aged men, elderly healthy men had significantly higher serum HE4 [median (1st and 3rd quartile): 53.7 (43.3–65.3) pmol/L vs. 67.2 (54.6–82.5) pmol/L; p=0.001] levels, but no such difference was found within the patient subgroups [109.9 (72.8–145.4) pmol/L vs. 126.8 (84.7–172.2) pmol/L; p=0.240] (Figure 1A).

No correlation was found between HE4 and creatinine levels (Spearman's ρ=−0.033, p=0.750) or eGFR (Spearman's ρ=−0.008, p=0.941) in the patient group, while there was a statistically significant negative correlation between HE4 and eGFR (Spearman's ρ=−0.278, p=0.005) in controls.

When the relationship between smoking and HE4 levels was analyzed, HE4 concentration was significantly higher in present smokers as compared to present non-smokers without malignancy [median (1st and 3rd quartile): 70.7 (55.6–93.3) pmol/L vs. 61.7 (46.9–73.1) pmol/L; p=0.039], but no such difference was observed among present smoking versus present non-smoking cancer patients [120.7 (87.1–146.4) pmol/L vs. 108.3 (66.6–168.0) pmol/L; p=0.378]. Overall, the present smoking and present non-smoking lung cancer patients had significantly higher HE4 concentrations as compared to their healthy counterparts (p<0.001) (Figure 1B, Table 2). The difference in HE4 levels between the healthy present smokers and non-smokers dissipated once the comparison was extended to the ever versus never smoking status (Table 2). Furthermore, the statistically significant difference in HE4 levels between cancer patients and healthy remained (p=0.001) even after adjusting for age, eGFR and smoking.

Comparison of the diagnostic characteristics of HE4 with classical tumor markers

In order to evaluate the discriminative power of HE4 and the other tumor markers ROC analysis was performed. The AUC for HE4 was 0.848 (95% CI 0.792–0.904) for differentiating all lung cancer patients from healthy controls, with a cut-off value of 97.6 pmol/L (sensitivity: 64.3%, specificity: 95.9%) (Figure 2A, Table 3). In the cancer cohort, the sensitivity of CEA, TPA, and Cyfra 21-1 was better or similar to that of HE4; furthermore, the specificity of HE4 was

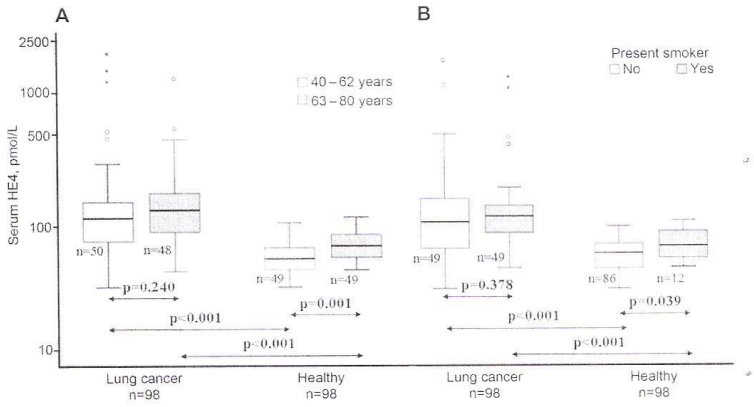


Figure 1 The level of serum HE4 in lung carcinoma and healthy subjects based on age (A) and present smoking habits (B). Using the median age of 62 years, the study populations were divided into a middle-aged (≤62 years) and an elderly (>62 years) subgroup. Elderly healthy men had significantly higher serum HE4 levels as compared to healthy middle-aged men (p=0.001), but no such difference was found within the patient subgroups (p=0.240) (A). HE4 concentration was significantly higher in present smokers as compared to present non-smokers without malignancy (p=0.039), however, no difference was observed among present smoking versus non-smoking cancer patients (p=0.378) (B).

comparable to that of the other markers (Figure 2A). HE4 correlated significantly with CEA (Spearman's ρ=0.235; p=0.020), CA 125 (Spearman's ρ=0.316; p=0.002), Cyfra 21-1 (Spearman's ρ=0.375; p<0.001), TPA (Spearman's ρ=0.384; p<0.001) and TK (Spearman's ρ=0.232; p=0.021), but not with NSE (Spearman's ρ=0.064; p=0.529) (data not shown).

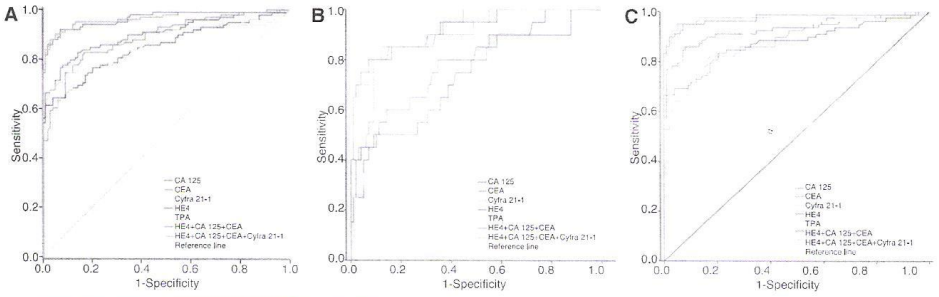
HE4 levels in different stages of lung cancer

HE4 concentrations were significantly higher in all clinical stages of the disease (p<0.05), with the most significant elevation (p=0.002) in stage IV, as compared to normal subjects (Figure 3A and Table 1). Similarly to CEA and CA 125, HE4 showed already statistically significant

Table 2 Smoking history of lung cancer patients and healthy controls.

	Lung cancer patients (n=98)	Healthy controls (n=98)
Never smokers, n (%)	4 (4%)	70 (71%)
HE4, pmol/L (median, 1st and 3rd quartile)	228.8[a] (72.9–1031.5)	62.3[b] (49.9–76.7)
Ever smokers, n (%)	94 (96%)	28 (29%)
HE4, pmol/L (median, 1st and 3rd quartile)	115.5[a] (80.7–146.1)	60.0[b] (45.2–75.4)
Pack-years, mean (range)	45 (8–135)	17 (1–58)
Present smokers, n (%)	49 (50%)	12 (12%)
HE4, pmol/L (median, 1st and 3rd quartile)	120.7[c,d] (87.1–146.4)	70.7[d,e] (55.6–93.3)
Pack-years, mean (range)	48.5 (13–100)	28.6 (20–38)
Duration of present smoking, years, mean (range)	37 (8–60)	22 (4–40)
Past smokers, n (%)	45 (46%)	16 (16%)
HE4, pmol/L (median, 1st and 3rd quartile)	108.1 (63.7–148.3)	47.0 (41.0–68.3)
Pack-years, mean (range)	41 (8–135)	14 (1–58)
Duration of past smoking, years mean (range)	33 (8–52)	19 (4–36)
Duration of non-smoking, years mean (range)	13 (1–40)	21 (2–60)
Present non-smokers, n (%)	49 (50%)	86 (88%)
HE4, pmol/L (median, 1st and 3rd quartile)	108.3[c,f] (66.6–168.0)	61.7[e,f] (46.9–73.1)

Category definitions: Ever smokers = present smokers + past smokers; Present smokers = ever smokers – past smokers; Past smokers = ever smokers – present smokers; Present non-smokers = never smokers + past smokers. Past and present smokers had quit or started smoking at least 1 year prior to study enrollment, respectively. [a]p=0.236; [b]p=0.540; [c]p=0.378; [d]p<0.001; [e]p=0.039; [f]p<0.001.



All lung cancer patients	Sensitivity	Specificity
CA 125 (35 kIU/L)	54.1%	100.0%
CEA (3.4 μg/L)	72.4%	90.8%
Cyfra 21-1 (3.3 μg/L)	63.3%	97.9%
HE4 (97.6 pmol/L)	64.3%	95.9%
TPA (75 U/L)	72.4%	86.7%
HE4 + CA 125 + CEA	91.8%	92.8%
HE4 + CA 125 + CEA + Cyfra 21-1	89.8%	94.9%

Lung cancer Stage I-II	Sensitivity	Specificity
CA 125 (35 kIU/L)	15.0%	99.0%
CEA (3.4 μg/L)	75.0%	90.8%
Cyfra 21-1 (3.3 μg/L)	25.0%	98.0%
HE4 (97.6 pmol/L)	40.0%	95.9%
TPA (75 U/L)	40.0%	86.7%
HE4 + CA 125 + CEA	80.0%	92.9%
HE4 + CA 125 + CEA + Cyfra 21-1	70.0%	93.9%

Lung cancer Stage III-IV	Sensitivity	Specificity
CA 125 (35 kIU/L)	64.1%	100.0%
CEA (3.4 μg/L)	71.8%	90.8%
Cyfra 21-1 (3.3 μg/L)	73.1%	98.0%
HE4 (97.6 pmol/L)	66.7%	95.9%
TPA (75 U/L)	82.0%	86.7%
HE4 + CA 125 + CEA	94.9%	92.9%
HE4 + CA 125 + CEA + Cyfra 21-1	94.9%	94.8%

Figure 2 Diagnostic value of HE4 and other classical tumor markers in all lung cancer patients (A), and in those in stages I–II (B), and stages III–IV (C).
ROC analysis was performed where the AUC for HE4 was 0.848 (95% CI 0.792–0.904) for differentiating all lung cancer patients from healthy controls at 97.6 pmol/L with better or similar sensitivity and specificity to that of CEA, TPA, Cyfra 21-1 (A). HE4 had a superior discriminative power [AUC 0.741 (95% CI 0.609–0.873)] as compared to Cyfra-21-1, NSE, TPA and TK in Stage I-II (B). In the early stages (B), the sensitivity of HE4 was low (40%), while in advanced stages (C) it was 66.7% with a specificity of 95.9%.

elevation in clinical stage I, and had a superior discriminative power [AUC 0.741 (95% CI 0.609–0.873)] as compared to Cyfra 21-1, NSE, TPA and TK in Stage I-II (Figure 2B, Tables 1 and 3). In the early (I–II) stages, the sensitivity was low (40%), while in advanced (III–IV) stages it was 66.7% with a specificity of 95.9% using the 97.6 pmol/L cut-off value (Figure 2B and C). We found two cancer patients in clinical stage I who were positive only for HE4, and negative for all the other tumor markers (data not shown).

Association between histology and HE4 levels

Next, we compared the levels of HE4 in patients with different histological subtypes of pulmonary malignancy. There was no significant difference in HE4 levels when comparing the two main histological types of lung cancer SCLC and NSCLC as follows: median [1st and 3rd quartile]: 120.7 (108.3–233.6) pmol/L vs. 113.9 (72.8–146.1) pmol/L; (p=0.153). Among NSCLC patients, individuals with any histology subtype showed significantly higher (p<0.001) HE4 levels compared

Table 3 Discrimination between healthy controls and lung cancer patients: ROC-AUC values.

	All lung cancer patients	Lung cancer St. I-II.	Lung cancer St. III-IV.
HE4	0.848 (0.792–0.904)[a]	0.741 (0.609–0.873)	0.875 (0.819–0.932)
CA 125	0.900 (0.855–0.945)	0.781 (0.666–0.895)	0.930 (0.888–0.973)
CEA	0.886 (0.893–0.932)	0.894 (0.821–0.967)	0.883 (0.832–0.935)
Cyfra 21-1	0.858 (0.802–0.914)	0.635 (0.478–0.792)	0.915 (0.868–0.962)
TPA	0.830 (0.770–0.891)	0.529 (0.364–0.694)	0.907 (0.861–0.954)
CEA-CA 125	0.943 (0.912–0.975)	0.894 (0.811–0.977)	0.956 (0.925–0.987)
CEA-CA 125-Cyfra 21-1	0.955 (0.928–0.981)	0.883 (0.807–0.959)	0.973 (0.949–0.997)
CEA-CA 125-TPA	0.950 (0.922–0.978)	0.872 (0.788–0.956)	0.970 (0.947–0.993)
CEA-CA 125-Cyfra 21-1-TPA	0.955 (0.928–0.982)	0.854 (0.763–0.945)	0.973 (0.948–0.997)
HE4-CA 125	0.933 (0.896–0.970)	0.857 (0.753–0.961)	0.953 (0.917–0.988)
HE4-CEA	0.930 (0.892–0.967)	0.908 (0.840–0.976)	0.935 (0.893–0.977)
HE4-Cyfra 21-1	0.899 (0.850–0.949)	0.729 (0.577–0.881)	0.943 (0.900–0.986)
HE4-CA 125-CEA	0.963 (0.937–0.990)	0.927 (0.865–0.989)	0.973 (0.945–1.000)
HE4-CA 125-CEA-Cyfra 21-1	0.964 (0.938–0.991)	0.914 (0.840–0.989)	0.977 (0.952–1.000)

[a]ROC-AUC value (95% confidence interval).

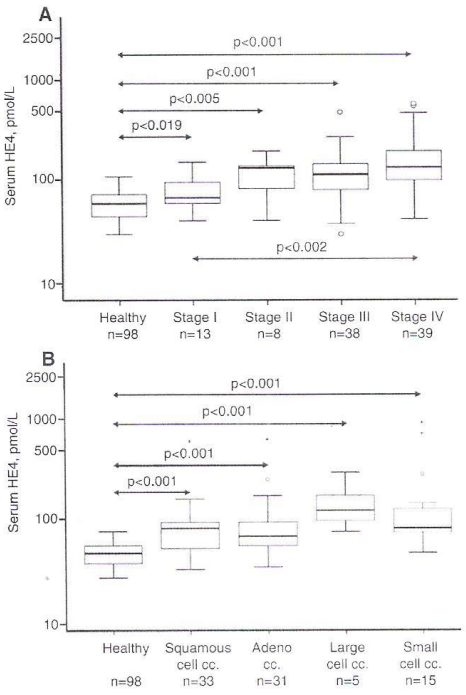


Figure 3 HE4 concentrations in lung cancer patients with different clinical stages (A) and histological subtypes (B).
HE4 levels were significantly higher in all clinical stages of the disease ($p<0.05$), with the most significant elevation ($p=0.002$) in stage IV, as compared to normal subjects (A). Patients with any histology subtype showed significantly higher ($p<0.001$) HE4 levels compared to the healthy cohort. The highest HE4 concentrations were measured in large cell carcinoma (B).

to the healthy cohort (Table 1, Figure 3B). The highest HE4 concentrations were measured in those who suffered from large cell carcinoma (n=5) [192.9 (128.8–408.4) pmol/L]. The ROC-AUC for HE4 was 0.827 (95% CI 0.760–0.895) for differentiating NSCLC patients from healthy persons with 74.3% sensitivity and 79.6% specificity at cut-off value of 79.65 pmol/L. In SCLC patients, ROC-AUC for HE4 was 0.939 (95% CI 0.866–1.000) with a sensitivity of 80.0% and a specificity of 89.8% at 104.6 pmol/L.

HE4 and the size and expansion of lung malignancy

Our patients had lung tumor with variable sizes [mean (range) 50.6 (13–130) mm]. A significant correlation was detected between HE4 values and the size of the tumor (Spearman's $\rho=0.227$, $p=0.030$). In 35% of our patients, the tumor demonstrated an invasion of the surrounding tissues based on CT/MRI examinations, while 65% of them had non-invasive cancer. Patients with regional tumor expansion (esophagus, pleura, pericardium, chest wall) showed higher HE4 concentrations as compared to those who did not [median (1st and 3rd quartile): 139.8 (96.8–235.4) pmol/L vs. 109.9 (69.1–141.3) pmol/L, $p=0.009$]. Patients presenting with distant organ metastasis had higher HE4 values as compared to those that had no metastasis [median (1st and 3rd quartile): 135.9 (97.8–192.9) pmol/L vs. 96.3 (63.5–141.8) pmol/L, $p=0.036$). Patients that did not present with general complaints (weakness, weight loss, cough, bloody sputum, chest pain or dyspnea) had significantly higher HE4 levels versus the healthy controls [median (1st and 3rd quartile): 113.5 (66.6–145.5) pmol/L vs. 62.2 (47.2–76.1) pmol/L, $p<0.001$], while there was no difference ($p=0.445$) in HE4 concentration between patients with and without complaints.

Prognostic value of HE4

Overall mean (range) survival was 254 (32–821) days for patients who died during the follow-up period (n=58, 59%). Among all cancer patients, 2-year overall survival rates in the HE4 positive (64.3%) group (at the cut-off value of 97.6 pmol/L) did not show statistically significantly worse prognosis than HE4 negative (35.7%) patients (Log rank test=0.233; Breslow test=0.687). Furthermore, there was no difference in survival rates upon extending this analysis (HE4 positive vs. HE4 negative) to the SCLC and NSCLC subgroups. In addition, there was no significant correlation between HE4 levels and the overall survival of the patients (Spearman's $\rho=-0.121$; $p=0.235$). Nonetheless, – independently of HE4 results – the SCLC patients had a significantly lower 2-year overall survival rate as compared to the NSCLC patients (Log rank test=0.004; Breslow test=0.004). When we examined the effect of HE4 on survival in patients in stage I/II (n=21), we did not see a significant difference either.

Combination of HE4 with classical tumor markers

Binary logistic regression was used to model the combination of HE4 with different classic tumor markers in discriminating cancer patients from healthy controls. The combination of two biomarkers could already enhance the

ROC-AUC values, but the highest discriminative power was achieved when HE4 was combined with CEA and CA 125 with or without Cyfra 21-1. In the case of early lung cancer (stage I–II) an AUC above 0.900 was observed only if HE4 was also included in the model (Table 3). The addition of Cyfra 21-1 enhanced AUC only very slightly (0.963 vs. 0.964), and the greatest AUC was observed with HE4+CEA+CA 125 combination [AUC: 0.927, (95% CI 0.865–0.989) sensitivity: 80.0%, specificity: 92.9%] in stage I–II cancer patients.

Discussion

Although a number of tumor markers are available in lung cancer that may support the diagnostics, histopathological discrimination, follow-up and prognostics of this disease [25–28], their role in the early detection of lung tumors is limited. Therefore, novel biomarkers with better clinical utility are much desired. Recently serum HE4 has been introduced for the routine diagnostics of ovarian cancer [29]. HE4 was first described in normal tissues such as the epithelium of epididymis, and the bronchial epithelium in the proximal respiratory tract [7]. Being a proteinase inhibitor, it plays variable physiological functions in the process of sperm maturation and some immune responses in the lung [17, 30]. However, HE4 may be overexpressed by several malignant tissues especially epithelial ovarian cancers [8, 10, 12, 31], but other gynecological [31, 32], gastrointestinal and pulmonary cancer cells [9, 12, 20, 31] were also shown to be positive for HE4 expression. As such, the evaluation of HE4 level in lung cancer seems to be reasonable.

In this study, we investigated HE4 concentrations in a large male cohort with different lung cancer subtypes, and compared these findings with healthy age-matched men. Age-dependency of HE4 levels was only evident in our healthy controls – as also reported previously [12, 33] – but no such tendency was observed in cancer patients. On the contrary, others have reported significant correlation between HE4 and age of patients [19]. Present smoking also affected HE4 levels in healthy individuals but not in patients. Furthermore, eGFR reduction – within the reference range – enhanced HE4 levels; this latter effect has been described [31, 34, 35]. This suggests that the malignant state per se overwhelms the effects of present smoking, reduced kidney function and age on HE4 levels.

There are only a handful of papers describing HE4 as a diagnostic and prospective marker for lung tumors, especially adenocarcinoma [12, 18–21]. Compared to these studies, the strength of our work is that it contains the most homogenous and well-characterized male patient and control cohort, and presents a comprehensive analysis of HE4 versus laboratory and clinical data. Escudero and colleagues [12] analyzed 77 patients with lung cancer and 101 controls, but no data are available about the sex, menopausal status, and the association of HE4 versus disease stages and survival. Similarly, in another study [21], no information was reported about the age of the patients and controls, furthermore, the sex of the control group was not mentioned. Two smaller studies [19, 24] described the difference of serum HE4 levels in lung cancer and controls, but the number of patients and controls studied was below 50, furthermore, information about the age, sex and menopausal status of the cohorts was missing.

In our lung cancer subjects, HE4 levels were significantly elevated as compared to the controls. HE4 values were comparable to those reported by Escudero et al. [12] who also used the same immunoassay, while other groups [19–21] using variable enzyme immunometric assays showed relatively lower HE4 levels in both cancer patients (50.7±24.5 pmol/L) and controls (23.8±4.5 pmol/L) [19]. Using the cut-off value of 97.6 pmol/L, we found that 64.3% of all patients were positive for HE4, while this rate was 60% for NSCLC and 80% for SCLC. In contrast, Yamashita and colleagues reported a lower percentage of HE4-positive subjects at a lower cut-off value (41.6% at 32.2 pmol/L; 43.2% at 50.3 pmol/L) [19, 21]. Others demonstrated even lower HE4-positive patients with NSCLC (29.3%) and SCLC (26.9%) [12]. It needs to be mentioned that Escudero et al. [12] did not calculate a new cut-off value for the lung cancer cohort, but used the postmenopausal cut-off value of >140 pmol/L for ovarian cancer. Of note, in our study we had two cancer patients who were positive for HE4, but negative for the other tumor markers.

Using ROC analysis we determined the discriminative power of HE4 and the classical tumor biomarkers. The optimal cut-off value for HE4 in the entire lung cancer cohort was calculated as 97.6 pmol/L, with an average (64.3%) sensitivity but a substantial (95.9%) specificity at the AUC value of 0.848. This AUC was similar to that reported previously (0.825 at 50.3 pmol/L cut-off) [21], while others found a higher AUC value (0.988) with an outstanding sensitivity (89.8%) and specificity (100%) at a cut-off value of 6.56 ng/mL (≈260 pmol/L) [20]. We suppose that the difference in AUC and discriminative features of HE4 are due to the distinct size and genetic background of studied populations, and the difference in the applied methods used for HE4 determination in previous studies. As yet, no comprehensive data on HE4 levels are available in European patient cohorts for further comparison.

Our patients demonstrated significantly increased HE4 levels at stage I, and further significant elevation was observed up to stage IV. In our study cohort, all end-stage patients were positive for HE4; this is in contrast to Yamashita and colleagues, who reported 50% HE4 positivity in this clinical stage [19]. We found that the levels of HE4 were related to the tumor load, and a significant correlation was found between HE4 values and the size of the primary tumor and the presence of lymph node metastasis. Surprisingly, two former studies did not observe statistically significant relationship between tumor size, vascular/lymphatic invasion and HE4 levels [19, 21]. In terms of nodal status, conflicting data are available; the same authors have reported a positive and a negative association with HE4 levels in two different papers [19, 21]. We did not find statistically significant difference in HE4 levels between the two main histological types of lung cancer similarly to Escudero et al. who reported HE4 levels in a few lung cancer individuals serving as controls to ovarian cancer patients [12]. Moreover, no significant difference was detected in HE4 concentration between the different subgroups of NSCLC, although the patients with large cell carcinoma had rather high values; this latter observation needs verification in a larger patient cohort.

The diagnostic power of HE4 was thoroughly compared to that of the CEA, Cyfra 21-1, NSE, and CA 125. CEA was considered as a reference biomarker in NSCLC, especially in advanced stages of squamous cell [5] and adenocarcinoma [36]. Similarly, we found CEA showing the highest AUC (0.917) among all measured tumor markers in our NSCLC patients. It was previously reported that CEA correlated significantly with HE4 [21]. A similar relationship between CEA and HE4 was also observed in our study. In NSCLC, the level of Cyfra 21-1 was previously found to be an independent prognostic marker in the earlier stages of NSCLC [6]. The AUC for Cyfra 21-1 was 0.852 in our patients, which was only slightly lower than previously published data (AUC=0.907) [5]. NSE is the first choice tumor marker in SCLC, but it can also be increased in around 25% of NSCLC cases [37]. We were able to confirm this finding, as AUC value of NSE (0.887) was the highest in SCLC. Additionally, the AUC for NSE was 0.700 in our NSCLC subjects, while others reported 0.740 [5].

Although individual tumor markers can provide reasonable sensitivity and specificity in the discrimination of healthy controls and cancer patients, the combined application may further enhance the power of these tests [38]. There are only a few studies that used this approach. The combination of classical serum tumor markers, acute phase reactants and different mediators of inflammation (cytokines, chemokines, adhesion molecules and proteinases) were applied in a panel of 2–6 [39–42] markers. The achieved diagnostic power was quite similar in these studies and our present data. The clearest advantage of the inclusion of HE4 in such a marker combination is the enhanced diagnostic power observed in stage I–II of the disease. Further advantage of our panel (HE4+CEA+CA 125) is its compact size; moreover, the measurement of commercially available routine tumor markers along with HE4 running on high throughput analyzers can be rather cost-effective.

When we studied the prognostic value of HE4, no significant correlation was seen between HE4 levels and the overall survival in either subgroup. In contrast, two studies [19, 20] showed – marginal and considerable – association between overall survival (but not disease-free survival) and HE4 in lung cancer patients. A recent study performed on large groups of NSCLC (n=190) and benign lung disease (BLD) (n=138, pulmonary tuberculosis and pneumonia) reported less impressive performance characteristics for HE4 in diagnosis of lung cancer, but observed a significant association of elevated HE4 with poor prognosis in terms of 3-year survivals [43]. Nonetheless, in our study the better diagnostic power of HE4 may be attributed to the comparison of healthy to lung cancer individuals; furthermore, the limited number of participants and follow-up period might explain the weak prognostic value of HE4.

Limitations of this study include its relatively small sample size, and heterogeneity of the lung cancer cohort, having a very small set of patients with SCLC. Furthermore, the use of patients with BLD as a reference group may have helped to address the issue of identifying lung cancer patients among those presenting with any pulmonary conditions.

In conclusion, our data indicate that HE4 is highly increased in male lung cancer patients – even without signs and symptoms of the disease – and shows a clear association with tumor size, and expansion, but does not differ significantly between histological subtypes of the malignancy. The combination of HE4 with CEA and CA 125 can further enhance the discrimination of cancer patients from controls even in stages I and II of the disease. In conclusion, serum HE4 may serve as a surrogate diagnostic marker of lung cancer in men.

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 funding played no role in the study design; in the collection, analysis, and

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