Human epididymal protein 4 (HE4) is a novel biomarker and a promising prognostic factor in ovarian cancer patients

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Summary

Purpose of investigation: The aim of this work was to compare serum concentrations of HE4 in patients with benign and malignant epithelial tumors and to determine the association of preoperative concentrations of HE4 with some clinicopathologic factors. Methods. We enrolled 94 patients, including 39 females with freshly diagnosed ovarian cancer. HE4 concentrations were measured with ELISA HE4 EIA assay from Fujirebio Diagnostics. Results. Serum concentrations of HE4 differed significantly in patients with ovarian cancer (324.1 pM) compared with benign epithelial tumors (26.1 pM; $p < 000.1$). There was also a significant difference between HE4 concentrations at diagnosis of ovarian cancer (324.1 pM) and in patients with complete clinical remission (23.3 pM; $p < 0.0001$). Patients with poorly differentiated tumors had significantly higher concentrations of HE4. Preoperative HE4 levels were higher in patients in whom relapse was noted and who died before the end of the two-year follow-up period. Conclusion. On the basis of these findings and reports in the literature it appears likely that HE4 can complement CA125 in the monitoring of therapy in ovarian cancer and may also serve for prognostication.

Key words: HE4; Ovarian cancer; Prognostic factors; Biomarkers.

Introduction

In spite of great efforts of medical specialists, researchers, and clinicians, ovarian cancer still remains the main cause of death among women with malignancies of the sex organs. Looking at the latest reports on ovarian cancer one may notice a specific "scientific race" for which the prominent goal is to accurately determine risk and prognostic factors, develop methods of early diagnosis, and improve therapeutic efficacy. During the last decade there emerged biomarkers discovered in the serum of patients with ovarian cancer but their sensitivity and specificity was never found to be superior to that of CA125 [1].

The human epididymal protein 4 (HE4) is among the very few biomarkers which have been studied recently and deemed suitable to play a leading role in the diagnostics and screening for ovarian cancer [2-5]. This protein was first discovered in the lining epithelium of the distal part of the epididymis [6] and was later identified exclusively in epithelial cells of various organs [7]. Physiologic expression of human HE4 is highest in the epithelium of the trachea, epididymis, female sex organs (oviducts, endometrium, and endocervix), and salivary glands [7, 8]. Expression of HE4 in the ovary is most noticeable in the metaplastic epithelium of Mullerian inclusion cysts [8]. Drapkin *et al*. found overexpression of HE4 in serous and endometrioid ovarian carcinomas but not in epithelial tumors of other organs (intestine, breast, lung, kidney or thyroid gland) [8]. It was inferred from these findings that cells of serous and endometrioid carcinomas of the ovary are capable of secreting HE4 to the extracellular space [8].

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The first article on the use of HE4 as an oncomarker in ovarian cancer was published by Hellstrom *et al*. [2] in 2003. During recent years, other researchers followed with a series of reports on the diagnostic importance of HE4 in ovarian cancer [9-12]. Unfortunately, little is known about the prognostic value of this epididymal protein. The present work was undertaken to compare serum concentrations of HE4 in malignant and benign epithelial tumors of the ovary with a focus on correlations of preoperative HE4 levels with other prognostic and clinicopathologic factors.

Material and Methods

Patients

Serum was obtained from 94 patients with epithelial tumors of the ovary seen at the Department of Gynecologic Surgery and Gynecologic Oncology of Adults and Adolescents and at the Outpatient Clinic of Gynecologic Oncology, Pomeranian Medical University. Some of our patients were referred to us due to the presence of an ovarian tumor. Transvaginal ultrasound was performed in each case to confirm the diagnosis of a tumor prior to surgical intervention. Initially, we enrolled 65 patients but this group was restricted to 56 patients with the histologic diagnosis of an epithelial tumor of the ovary (we excluded patients with endometriosis and other benign non epithelial tumors). These patients were divided into two groups:

A) patients with ovarian cancer (n = 39);

B) patients with benign epithelial tumor (n = 17).

The second part of our patients (n = 38) were admitted to the hospital for second-look laparoscopy in ovarian cancer. Based on histopathological results of clippings we assigned these patients to groups:

C) with histologically confirmed disease free survival (n = 16);

D) with relapse or residual disease (n = 22).

In some patients of group D, relapse was confirmed with computed tomography (CT). All patients provided informed consent to participate in the study.

The groups (A, B, C, D) were compared as to concentrations of HE4 and CA125, and a detailed analysis of data in group A was done. Comparisons were performed in the whole group, in the subgroup with serous cancer, and in patients with FIGO III clinical stage. We analyzed HE4 and CA125 levels depending on the FIGO stage and cellular differentiation. Prognostic usefulness of HE4 was studied by correlating preoperative levels with the fact of disease-free survival (DFS) (no symptoms from the last chemotherapy session to relapse), time of DFS in months, relapse (only patients with complete remission, excluding those resistant to chemotherapy), two-year survival, and death during follow-up. All patients were treated at our Department between 2006 and 2008 and the final verification of clinical data concerning survival was done in January 2011.

Serum collection

Blood was collected at admission, one day before surgery, and centrifuged. Aliquots of serum were stored until analysis.

Marker assays

CA125. Assays were performed at the Laboratory of Hormones and Oncomarkers of the Department of Gynecologic Surgery and Oncology or at the Central Laboratory of the Independent Public Hospital of the Pomeranian Medical University. CA125 was determined in most patients visiting our Department or Clinic, using commercially available test kits from Abbott. The upper normal value was 35 U/ml.

HE4. Serum HE4 concentrations were measured using the HE4 EIA assay from Fujirebio Diagnostics. This solid-phase, non-competitive immunoassay based on the direct sandwich technique was carried out according to the manufacturer's instructions. Appropriate controls were within the ranges provided by the manufacturer. The limit of detection (LoD) corresponded to the upper limit of the 95% confidence interval and represented the lowest concentration of the HE4 antigen that can be distinguished from zero. LoD of the HE4 kit was calculated to be < 2.5 pM and the normal upper limit was taken as 150 pM given by the manufacturer.

Statistical analysis

Statistics were performed using STATISTICA 9.1 PL software. Means were compared with the nonparametric Mann-Whitney U test or the Kruskal-Wallis test. Qualitative variables were analyzed with contingency tables and the chi-square test. Parametric linear correlations and nonparametric Spearman's and Kendall's rank correlations were studied. Receiver operating characteristic (ROC) curves were obtained and the area under the curve (AUC) was calculated with a 95% confidence interval. The sensitivity and specificity values for some clinicopathologic parameters were determined in group A. AUC = 1 (or 0) corresponded to the ideal ROC curve and the test was deemed useful for AUC ≥ 0.5. The level of significance was taken as $p < 0.05$.

Results

Patient data of group A are presented in Table 1. All the patients from group A were treated with debulking surgery and taxane-platinum-based chemotherapy.

Group B included patients with benign cystadenomas: eight with serous tumors, six with mucinous tumors, and three with cystadenofibroma. The mean age in this group was 48.5 years. There were ten postmenopausal patients with a mean age of 62.3 years and mean HE4 concentration of 40.6 pM (9.39-101.9) and seven patients in reproductive age with a mean age of 27.8 years and mean HE4 concentration of 5.35 pM (2.9-10.6). Five mucinous tumors were diagnosed in postmenopausal patients – their mean HE4 concentration was 58.45 pM (10.41 – 101.9). For serous tumors, the mean HE4 concentration was 22.9 pM (2.9-85.8). While none of the patients in group B had HE4 concentrations above the normal range, 29.4% of them had elevated CA125 levels. There was a linear correlation (R = 0.527161, $p < 0.05$) and a nonparametric correlation (Spearman's, Kendall's, $p < 0.005$) between concentrations of CA125 and HE4 in group B.

Group C consisted of patients with histologic evidence of clinical remission. There were no tumor cells in histo-

Table 1. — *Characteristics of the patients (group A).*

	n = 39 (total) Mean age = 53.6 [32 = 79]	%
Premenopausal		
Mean age 44.2 [32-50]	14	35.9
Postmenopausal		
Mean age 60.64 [50-79]	25	64.1
BMI > 30	29	69.2
BMI < 30	12	30.8
FIGO I	10	25.6
FIGO II	5	12.8
FIGO III	23	59
FIGO IV	1	2.6
Grade 1	6	15.4
Grade 2	13	33.3
Grade 3	20	51.3
Serous	29	74.4
Mucinous	2	5.1
Endometrioid	2	5.1
Clear cell	4	10.3
Undifferentiated	2	5.1

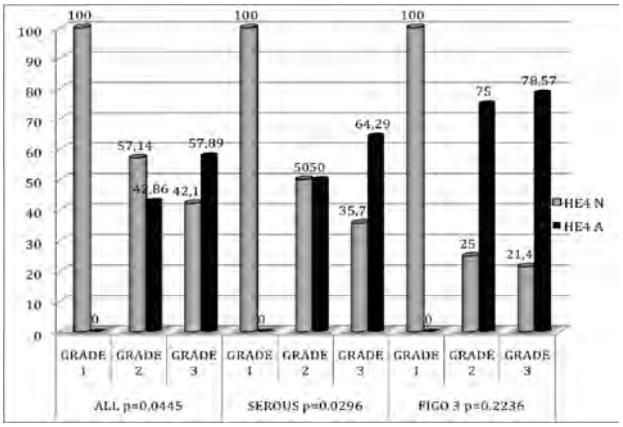

Figure 1. — Percentage of normal and elevated HE4 levels in serum of patients in group A (all, serous tumors, FIGO III) depending on cellular differentiation of the tumor.
*HE4 N (normal concentration of HE4); HE4 A (above-normal concentration of HE4).

Fig. 2a

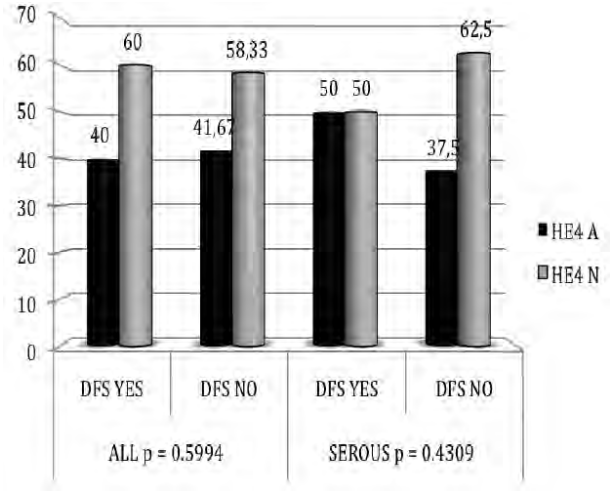

Fig. 2b

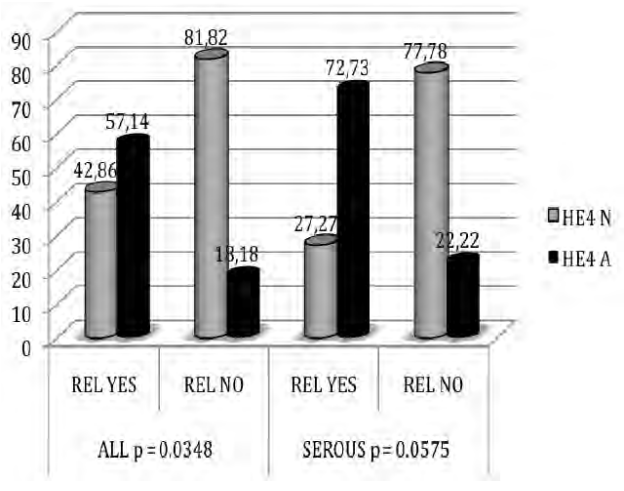

Fig. 2c

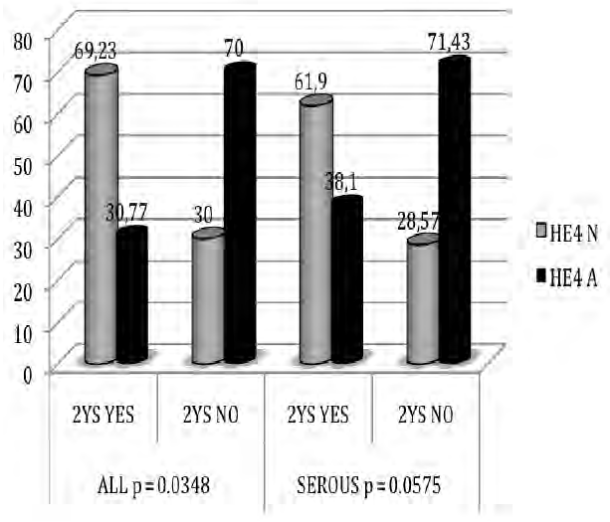

Fig. 2d

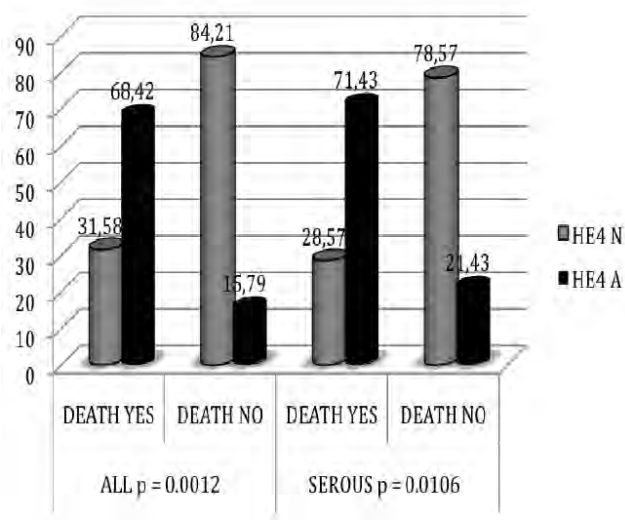

Figure 2. — Percentage of normal and elevated HE4 levels in serum of patients in group A (all, serous tumors) depending on 1) disease-free survival, 2) relapse, 3) two-year survival, 4) death.
(zamiast 1, 2, 3, 4 powinno by A, B, C, D).
*HE4 N (normal concentration of HE4); HE4 A (above-normal concentration of HE4).

logic specimens, no symptoms of disease, and no signs of any active neoplastic process in CT. All patients had previously undergone optimal surgery and standard chemotherapy based on platinum analogs. The mean age in this group was 53 years (24-75). In 11 patients with serous cancer, the mean HE4 concentration during remission was 32.59 pM (4.54-106.3). In the remaining patients (2 endometrial, 1 mucinous, 1 clear cell, and 1 transitional cell cancer), the mean HE4 concentration was 13.7 pM (4.6-36.9). None of the patients had elevated CA125 or HE4 levels, which was in strict correlation with their clinical status.

Group D comprised patients with previously diagnosed ovarian cancer, in whom laparoscopy and histology disclosed the presence of active disease or who presented with a mass in the pelvis or abdomen appearing during diagnostic imaging to be the sequella of the primary diagnosis. Mean age in this group was 56 years [43-75]. There were 19 cases of serous cancer. Differentiation grade of the cancer was G3 in 13 cases (mean HE4 = 361.6 pM (33.8-1558.9), mean CA125 = 1149 U/ml (23 -6000) and G1 or G2 in eight cases (mean HE4 = 171.8 (7.9-700.4), mean CA125 = 158.7 U/ml (7.06-1155.48)). Taking 150 pM as the upper normal limit for HE4, we found that seven patients (33.3%) had elevated HE4 levels at the time of relapse or residual disease was diagnosed. This subgroup increased to 16 patients (76%) when we reduced the upper limit to 70 pM as suggested by Moore *et al*. [5]. As regards CA125, the upper normal limit of 35 U/ml was exceeded in 11 patients (52%).

Concentrations of HE4 and CA125 in the groups are presented in Tables 2 and 3. Significantly higher concentrations of HE4 and CA125 were found in patients with malignant epithelial tumors at the time of diagnosis

Table 2. — *Concentrations in serum of HE4 and CA125 in ovarian cancer patients depending on stage and in patients with benign epithelial tumors*.

Group	A Mean range	B Mean range	*p*	A Mean range	C Mean range	*p*	A Mean range	D Mean range	*p*
HE4 [pM]	324.1 [2.5-1574.9]	26.1 [0.0 -101.9]	< 0.0001	324.1 [2.5-1574.9]	23.25 [0.0-106.33]	< 0.0001	324.1 [2.5-1574.9]	289.5 [7.91-1558.9]	NS
CA125 [U/ml]	1961.9 [7.38-22982]	35.3 [3.86-186.72]	< 0.000005	1961.9 [7.38-22982]	16.1 [2.7-53.7]	< 0.000005	1961.9 [7.38-22982]	769.1 [5.38-6000]	< 0.05

Table 3. — *Concentrations in serum of HE4 and CA125 in ovarian cancer patients during remission and relapse and in patients with benign epithelial tumors*.

Group	B Mean range	C Mean range	*p*	B Mean range	D Mean range	*p*	C Mean range	D Mean range	*p*
HE4 [pM]	26.1 [0.0 -101.9]	23.25 [0.0-106.33]	NS	26.1 [0.0 -101.9]	289.5 [7.91-1558.9]	< 0.00005	23.25 [0.0-106.33]	289.5 [7.91-1558.9]	< 0.00005
CA125	35.3 [3.86-186.72]	16.1 [2.7-53.7]	NS	35.3 [3.86-186.72]	769.1 [5.38-6000]	< 0.01	16.1 [2.7-53.7]	769.1 [5.38-6000]	< 0.0005

Table 4. — *Concentration in serum of HE 4 and CA 125 in ovarian cancer patients depending on FIGO stage*.

	FIGO I	FIGO II	*p*	FIGO I	FIGO III	*p*	FIGO II	FIGO III	*p*
					WHOLE GROUP A				
HE4 [pM] mean range	46.07 (0.0-136.31)	46,82 (20.39-102.07)	NS	46.07 (15.64-76.59)	513.48 (0.0-1574.89)	$p < 0.0005$	46,82 (20.39-102.07)	513.48 (0.0-1574.89)	p = 0.0578
CA 125 [U/ml] mean range	176.8 (15.81-500)	2859.21 (7.38-21442)	NS	176.8 (53.51-300.1)	2018.26 (49.22-22982)	$p < 0.005$	2859.21 (7.38-21442)	2018.26 (49.22-22982)	p = 0.0741
					SEROUS ONLY				
HE4 [pM] mean range	26.16 (0.0-79.11)	28.64 (20.39-35.17)	too few cases for statistical analysis	26.16 (0.0-79.11)	537.97 (20.67-1310.69)	$p < 0.001$	28.64 (20.39-35.17)	537.97 (20.67-1310.69)	too few cases for statistical analysis
CA 125 [U/ml] mean range	140.14 (15.81-445)	221.37 (16.55-595.34)		140.14 (15.81-445)	2383.97 (49.23-22982)	$p < 0.001$	221.37 (16.55-595.34)	2383.97 (49.23-22982)	

(group A) and at the time of relapse or disclosure of residual disease (group D), compared with patients with benign epithelial tumors (group B) or with ovarian cancer during complete clinical remission (group C). There was close to a significant difference between patients with FIGO II and FIGO III in group A as regards HE4 (p = 0.0578) and CA125 (p = 0.0741) (Table 4). Qualitative analysis evidently disclosed that higher concentrations of HE4 (above 150 pM) were associated with high clinical stage of the tumor. We could clearly see that HE4 levels correlated inversely with cellular differentation. Poorly differentiated tumors demonstrated higher HE4 values in all groups and subgroups although statistical significance was disclosed only between G1 and G3 (Table 5). Univariate qualitative analysis revealed that normal HE4 concentrations in serum correlated with a highly mature form of cancer in the whole group and in the subgroup of serous cancer. The tendency was similar in high-grade cancer, albeit without statistical significance.

There was no correlation betweem serum concentrations of HE4 or CA125 and disease-free survival (DFS) (Table 6). Instead, there was a significant correlation between HE4 levels and duration of DFS (Figures 5 and 6). Concentrations of HE4 and CA125 correlated with relapse (Table 6): this was demonstrated by comparing means (Table 6) and carrying out qualitative analysis (Table 5). Table 6 and Figure 2 present the correlation between serum concentrations of HE4 and two-year survival or death during follow-up. Two-year survival was more frequent among patients with lower initial concentrations of HE4. Significance was noted when means were compared (242.72 pM in patients who survived vs 445.78 pM in patients who did not survive two years). The same was shown in qualitative analysis: 69.2% patients who survived two years had normal levels of HE4 whereas 70% patients who did not survive had HE4 levels above 150 pM. Kaplan-Meier curves revealed a statistically significant correlation between survival and

Table 5. — *Concentration in serum of HE 4 and CA 125 in ovarian cancer patients depending on grade*.

	GRADE 1	GRADE 2	*p*	GRADE 1	GRADE 3	*p*	GRADE 2	GRADE 3	*p*
					WHOLE GROUP A				
HE4 [pM] mean/range	36.35 (0.0-79.11)	288.45 (4.63-911.96)	NS	36.35 (0.0-79.11)	441.24 (0.0-1574.89)	$p < 0.05$	288.45 (4.63-911.96)	441.24 (0.0-1574.89)	NS
CA 125 [U/ml] mean/range	2910.01 (7.38-21442)	703.18 (49.22-1096)	NS	2910.01 (7.38-21442)	2125.89 (27.83-22982)	$p < 0.05$	703.18 (49.22-1096)	2125.89 (27.83-22982)	NS
					SEROUS ONLY				
HE4 [pM] mean/range	36.35 (0.0-79.11)	401.21 (4.64-911.96)	NS	36.35 (0.0-79.11)	437.27 (20.39-1310.69)	$p < 0.01$	401.21 (4.64-911.96)	437.27 (20.39-1310.69)	NS
CA 125 [U/ml] mean/range	1173.43 (15.81-94.16)	841.81 (49.23-2096)	NS	1173.43 (15.81-94.16)	2503.26 (27.83-22982)	$p < 0.01$	841.81 (49.23-2096)	2503.26 (27.83-22982)	NS

Table 6. — *Concentration in serum of HE 4 and CA 125 in ovarian cancer patients depending on disease-free survival (DFS), two-year survival (2YS) and relapse*.

	HE4 [pM]	*p*	CA 125 [U/ml]	*p*	HE4 [pM]	*p*	CA 125 [U/ml]	*p*	HE4 [pM]	*p*	CA 125 [U/ml]	*p*
	ALL PATIENTS				SEROUS ONLY				FIGO III ONLY			
DFS YES	313.66 (0.0-1310.69)	NS	2386.22 (7.38-22982)	NS	375.27 (0.0-1310.69)	NS	2188.39 (15.81-22982)	NS	606.99 (12.58-1310.69)	NS	3068.59 (59.71-22982)	NS
DFS NO	250.43 (0.0-590.13)	NS	1009.38 (49.22-5887)	NS	255.54 (20.67-590.13)	NS	1153.61 (49.22-5887)	NS	303.66 (0.0-590.13)	NS	1032.56 (49.22-5887)	NS
RELAPSE YES	499.33 (4.64-1310.69)	$p < 0.05$	882.18 (59.71-4882.1)	$p < 0.05$	617.78 (4.63-1310.69)	$p < 0.05$	1019.12 (98.1-4882.1)	$p < 0.01$	too few cases for statistical analysis		too few cases for statistical analysis	
RELAPSE NO	77.36 (15.81-273.31)	$p < 0.05$	503.72 (15.81-3743.5)	$p < 0.05$	78.86 (0.0-273.31)	$p < 0.05$	549.39 (15.81-3743.5)	$p < 0.01$				
2YS YES	245.72 (0.0-1310.69)	$p < 0.05$	892.45 (15.81-5887)	$p < 0.05$	288.22 (0.0-1310.69)	p = 0.06719	1028.85 (15.81-5887)	NS	448.56 (12.58-1310.69)	NS	1468.59 (59.71-5887)	NS
2YS NO	445.78 (35.17-1046.12)	$p < 0.05$	748.43 (145-1865)	$p < 0.05$	499.57 (35.17-1046.12)	p = 0.06719	820.56 (145-1865)	NS	597.94 (320.11-1046.12)	NS	714.11 (145-1837.02)	NS

HE4 levels (Figures 3 and 4). Using ROC curves to determine sensitivity and specificity of the method in group A, we found the following probabilities of occurring relapse as 0.753, DFS 0.483, two-year survival 0.754, and death 0.163 for HE 4. For CA125, the probabilities were: 0.778, 0.557, 0.675, and 0.667, respectively. We repeated this analysis for patients with serous tumors in group A and found the following areas under ROC curves for HE4: relapse 0.828, DFS 0.5, two-year survival 0.5, and death 0.862. The respective values in the case of CA125 were 0.822, 0.448, 0.356, and 0.658. Some of these data are shown in Figure 7.

Discussion

There are reports suggesting that various histologic subtypes of ovarian cancer are in fact different nosologic units revealing a different natural history of onset, clinical course, and prognosis. Biomarkers should be helpful in the determination of the precise biology of ovarian epithelial tumors and in consequence should have an impact on individualized therapies [13]. During recent decades, the sensitivity and specificity of some oncomarkers in serum have been measured in patients with ovarian cancer. None of them performed any better than CA125 [1]. Unfortunately, elevated levels of this antigen are observed in malignancies of organs other than the ovary and in non-oncologic diseases, limiting the usefulness of CA125 as far as preoperative diagnosis of ovarian tumors is concerned [14, 15].

HE4 is one of the most interesting and promising novel biomarkers which may be useful for routine management of patients with ovarian cancer and in the differential diagnosis of pathologies of the adnexes [16, 17]. Huhtinen *et al*. [16] demonstrated that combined measurements of HE4 and CA125 in serum are much more valuable than determinations of each of these markers alone. For example, elevated levels of CA125 and HE4 in patients with a pathology of the ovary found at ultrasonography will suggest an ovarian malignancy whereas elevated serum level of CA125 and normal level of HE4 points more to endometriosis or to another benign lesion. Vice versa, elevated HE4 and normal CA125 may rather be associated with a tumor of a female sex organ other than the ovary (e.g. endometrial cancer) [16].

Most studies on HE4 in ovarian cancer carried out in recent years have focused on the diagnostic importance of this oncomarker and on prediction of ovarian cancer [3,5,9]. It was demonstrated that 32% of ovarian cancer cases reveal expression of HE4 and no expression of CA125. Logically, determination of both markers should markedly improve prediction of ovarian cancer [1]. In

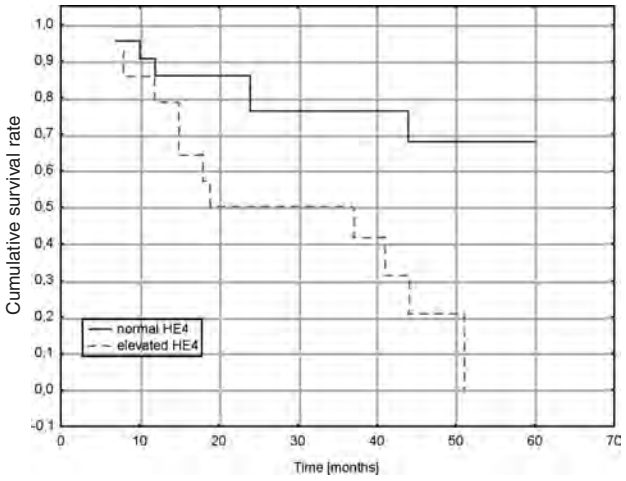

Figure 3. — Kaplan-Meier estimates of overall survival in group A depending on HE4 level in serum.
*HE4 N (normal concentration of HE4); HE4 A (above-normal concentration of HE4).

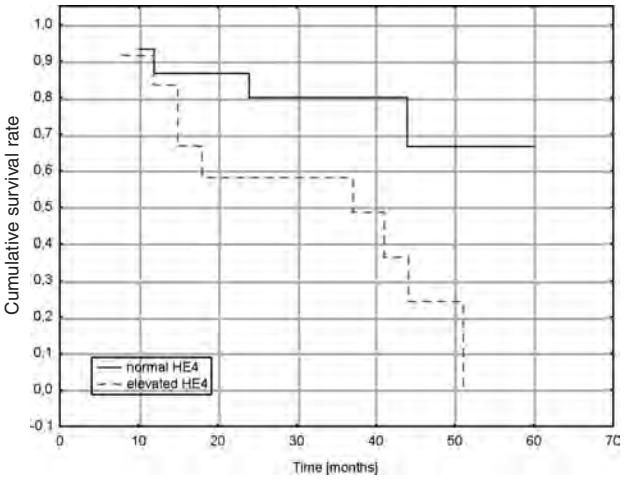

Figure 4. — Kaplan-Meier estimates of overall survival of patients with serous tumors in group A depending on HE4 level in serum.
*HE4 N (normal concentration of HE4); HE4 A (above-normal concentration of HE4).

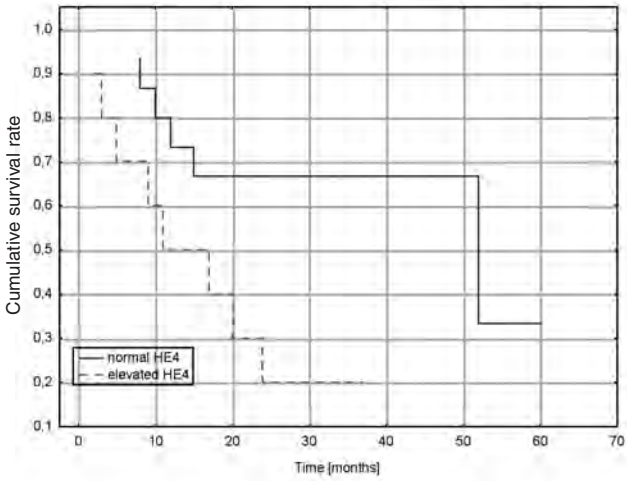

Figure 5. — Kaplan-Meier estimates of disease-free survival (DFS) in all patients of group A depending on HE4 level in serum.
*HE4 N (normal concentration of HE4); HE4 A (above-normal concentration of HE4).

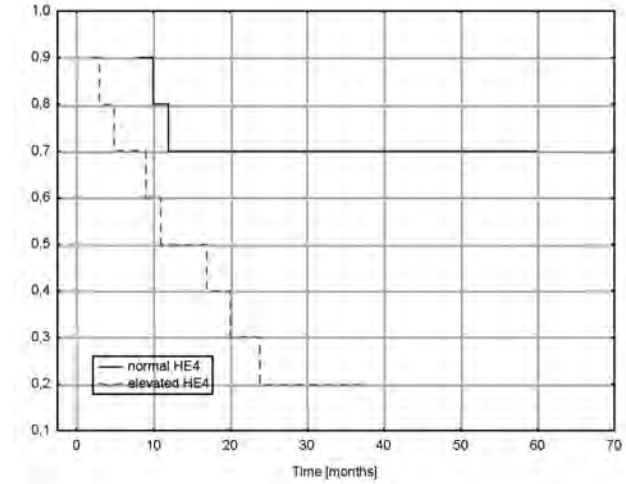

Figure 6. — Kaplan-Meier estimates of disease-free survival of patients with serous tumors in group A depending on HE4 level in serum.
*HE4 N (normal concentration of HE4); HE4 A (above-normal concentration of HE4).

June 2010, the American FDA (Food and Drug Administration) approved the HE4 test from Abbott for monitoring treatment of ovarian cancer patients. Shah *et al*. [12] reported that the sensitivity of differentiating ovarian cancer from benign lesions of the adnexes in patients at an average population attributable risk is 58.8% for CA125 and 61.8% for HE4. These figures rose to 79.4% and 80.4%, respectively, when ovarian cancer patients were compared with healthy individuals. A minor increase in sensitivity was observed for women at an increased population attributable risk. Hevrilesky *et al*. [4] demonstrated that the sensitivity of HE4 and CA125 for early diagnosis of ovarian cancer is 82.7% and 45.9% respectively, and 92.5% and 58.5% respectively, for advanced cancer. The specificity of CA125 and HE4 was 98.2% and 86.4%, respectively. It was suggested that high sensitivity and specificity warrants the use of the test for screening of the general population [4]. Moore *et al*. [5] found that the sensitivity in ovarian cancer was 72.9% for HE4, 43.3% for CA125, and 76.4% CA125 and HE4 combined. Taking the upper normal limit for HE4 as 150 pM, we found above-normal levels of this marker in 43.6% of patients with freshly diagnosed ovarian cancer and in 38.1% with relapse. Normal levels of HE4 were confirmed in all patients with benign lesions and in patients during remission. However, when we set the upper normal limit at 70 pM after Moore *et al*. [5], HE4 was elevated in 63.2% of patients at diagnosis of ovarian cancer and in 76% of patients at relapse. One may rightly ask whether the upper normal limit given by the manu-

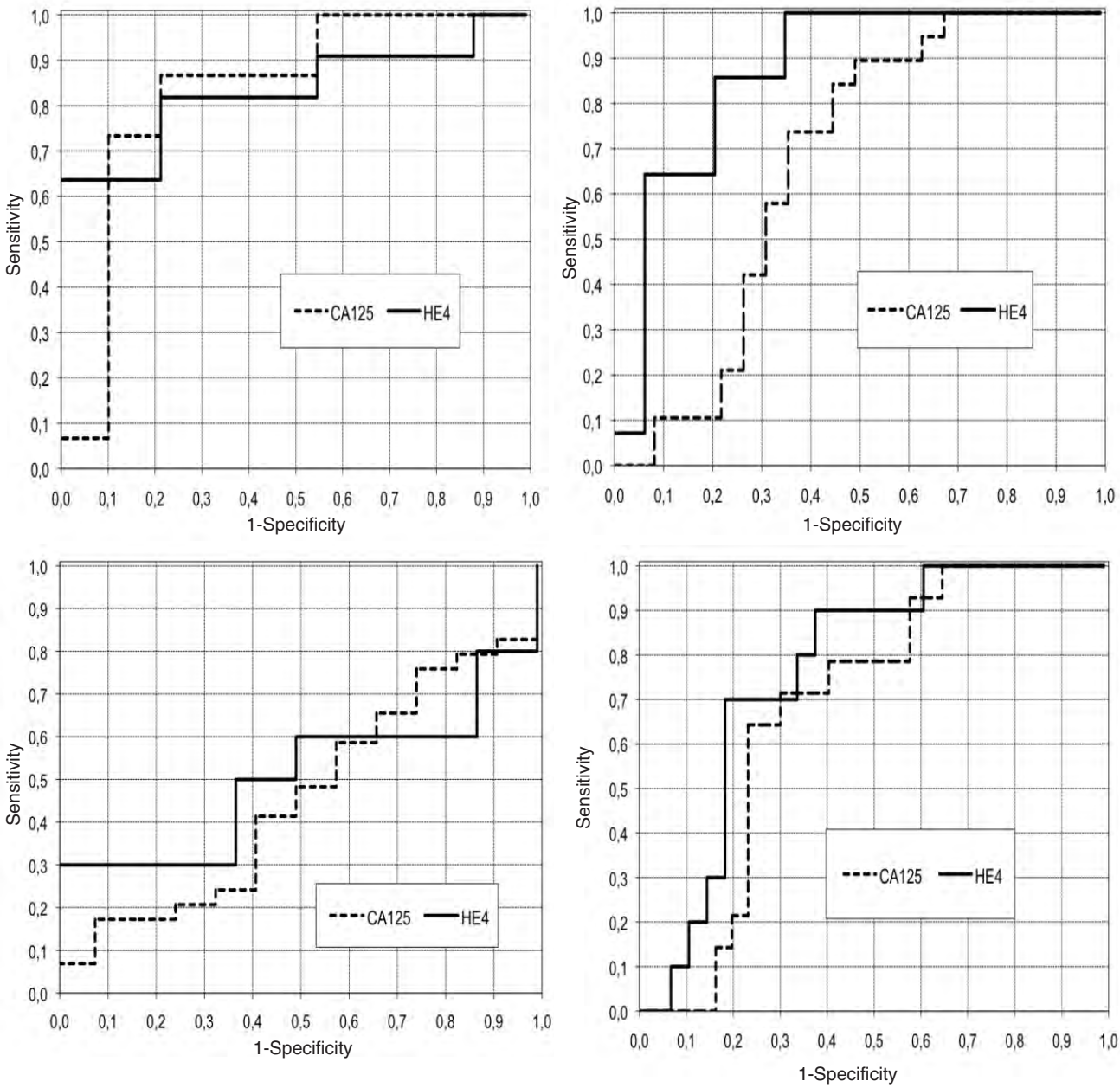

Figure 7. — ROC curves for CA125 and HE4 in patients with malignant epithelial tumors of the ovary.
A) relapse, B) death, C) disease-free survival, D) two-year survival.

facturer conforms with the pattern of HE4 in healthy women and as a consequence, whether the sensitivity of the test for diagnosing ovarian malignancies is reduced at this limit. Based on our results, we believe that the normal range for HE4 awaits verification. Anastasi *et al*. [18] demonstrated that mean values of HE4 do not change in a statistically significant manner during the menstrual cycle, ranging from 37.5 pM to 46.6 pM in women before the age of 35 years and from 39.7 pM to 45 pM in older women, not exceeding 70 pM even when two standard deviations were added. Moore *et al*. [5] reported that the mean HE4 concentration was 58.6 pM in women with benign ovarian pathologies, 70.8 pM during postmenopause, and 51.2 pM during premenopause. Wang *et al*. [19] found 34.1 pM as the mean value for HE4 in healthy women and 39.1 pM in benign tumors. In the present study, the mean value for HE4 in patients with benign ovarian pathologies (group B) was 26.1 pM (95% CI = 8.5-43.8), again markedly below the assumed upper normal limit of 150 pM. In no patient of this group did the concentration of HE4 exceed 70 pM.

HE4 and CA125 are often used in specific algorithms aimed at early detection of ovarian cancer [3, 10, 20, 21] which take into account high or average population

attributable risk, hormone status (pre- or postmenopause), and diagnostic imaging findings. Risk of Ovarian Malignancy Algorithm (ROMA) developed by Moore *et al*. [5] in 2010 is one of these algorithms. According to these authors, its sensitivity is 94.3%, clearly above 84.6% for the previously used Risk of Malignancy Index (RMI) algorithm. An interesting study was done by Anderson *et al*. [21] who found that HE4 and CA125 levels in serum are able to predict ovarian cancer several years prior to the final diagnosis. Based on these results it appears that the greatest benefit could be expected for BRCA1 mutation carriers who are candidates for preventive salpingo-oophorectomy, in whom the timing of the operation could be optimized. We were able to demonstrate that HE4 is markedly increased in patients with high FIGO clinical stage. Normal levels of HE4 were observed in all patients with FIGO I and FIGO II, whereas above-normal levels were found in 73.9% of patients with FIGO III of group A and in 76.5% of patients with serous cancer. These differences were statistically significant. We also found that all patients with highly mature forms of cancer had normal levels of HE4 as opposed to the finding of above-normal levels in most patients with poorly differentiated cancer (Figure 1). In this case, the difference was also statistically significant. In line with our results, Van Gorp *et al*. [9] observed differences in HE4 concentrations depending on the stage of the tumor but failed to find statistically significant differences between grades 1, 2, and 3.

Anastasi *et al*. [22] reported in 2010 that HE4 may serve as an early biomarker of relapse but their study was done in eight ovarian cancer patients only, in whom complete remission was achieved after the first chemotherapy cycle. During follow-up, HE4 and CA125 levels were increased in five patients. The increase in HE4 preceded the increase in CA125 by five to six months. In the present study, the upper normal limit of 70 pM for HE4 was exceeded in 76% of patients with relapse of ovarian cancer (group D) while only 52% of them had above-normal levels of CA125.

The basic objective of the present study was to determine whether one of the most promising novel biomarkers for ovarian cancer may also serve as a prognosticator. So far, there are only two studies discussing prognostic significance of human epidydymis protein 4 in epithelial ovarian cancer [23, 24]. Results of Paek and colleagues [23] demonstrated that elevated serum HE4 level was related to the advanced stage of ovarian cancer and was a poor prognostic factor for PFS. Steffensen *et al*. [24] found HE4 to be a strong independent indicator of worse prognosis in epithelial ovarian cancer unlike CA 125 and HER2. Yamashita *et al*. [25] discussed the prognostic importance of HE4 expression in 137 female patients with pulmonary adenocarcinoma and found that expression heralded a poor prognosis and indicated a shortening of DFS and overall survival times. We observed that HE4 levels at diagnosis did not correlate with the prognosis of complete remission but were implicated in the duration of remission as seen from the Kaplan-Meier curves presented in Figures 3 and 4. The duration of complete remission was significantly longer in patients with normal HE4 concentrations in serum. In other words, relapse was less frequent in patients with normal HE4 levels. Elevated HE4 concentrations in group A were found in 57.1% of relapse cases and in 72.7% of patients with serous cancer (Figure 2). Similar figures were observed for two-year survival and moreover, there was a clear tendency to elevated HE4 levels in ovarian cancer patients who did not survive for at least two years from diagnosis. Normal HE4 concentrations in two-year survivors of group A were disclosed in 69.2% of the whole group and in 71.43% of serous cancer cases ($p = 0.0348$ and $p = 0.0575$, respectively). Using ROC curves we were able to confirm that HE4 performs as well or better than CA125 as a biomarker for prognostication in ovarian cancer.

It can be inferred from the literature on HE4 patterns and from our present results that HE4 may be a valuable supplement to CA125 for the monitoring of therapy in ovarian cancer and may also serve as an important prognostic factor. It is too early now for final conclusions until the results can be corroborated in a large group of patients. In spite of the growing knowledge on the potential applications of HE4 in clinical practice, there remain "gaps" which need to be filled in quickly.

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