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Original Articles

Is human epididymis protein 4 an effective tool for the differential diagnosis of benign and malignant endometrial tumours?

L. Minar[1], I. Klabenesova[2], E. Jandakova[3], F. Zlamal[4], J. Bienertova-Vasku[4]

[1] Department of Obstetrics and Gynaecology, Faculty of Medicine, Masaryk University Brno and University Hospital Brno, Brno
[2] Department of Biochemistry, University Hospital Brno, Brno; [3] Department of Pathology, University Hospital Brno, Brno
[4] Department of Pathological Physiology, Faculty of Medicine, Masaryk University Brno, Brno (Czech Republic)

Summary

Purpose of investigation: This study was designed to evaluate the use of human epididymis protein 4 (HE4) as a biomarker in the differential diagnosis of malignant and benign endometrial tumours. *Materials and Methods:* The study, conducted between July 2009 and June 2014, included a total of 150 patients with endometrioid adenocarcinoma and a control group of 150 patients with benign endometrial lesions. The serum of all patients was analyzed with respect to HE4 and CA125 levels. The median and ranges of serum levels were determined in relation to histological results. The statistical analysis procedure employed in this study utilized logarithmic-transformed values of biomarkers and logistic regression. *Results:* An analysis of two groups of patients with different histologies yielded a statistically significant difference (p-value < 0.05) only in the case of HE4, in which case a cut-off value of 48.5 pmol/l resulted in an achieved sensitivity of 87.8%, a specificity of 56.6%, and a negative predictive value of 81.1%. *Conclusion:* In combination with clinical and ultrasound findings, HE4 could help with the differentiation of prognostically varied patient groups as well as with the decision-making process associated with the development of individual treatment plans. However, the optimal cut-off for HE4 has not been established yet and further studies are needed.

Key words: Benign endometrial tumours; Endometrial cancer; Human epididymis protein 4.

Introduction

Endometrial cancer is the most common malignancy of the female genital tract in the world and the seventh most common cause of cancer-related death in women in Western and Central Europe [1]. Prognosis for this group of gynaecological malignancies is relatively favourable, as long as they are diagnosed in the first stage, which is currently the case in almost 75% of patients [2]. The endometrioid histological type of endometrial cancer is the prevalent type of this malignancy (over75 % of all diagnosed cases) [3].

Despite relatively favourable prognosis, research has focused on the detection of a marker for efficient and economical screening and for early disease diagnosis. Such a marker could benefit women at increased risk (e.g. breast cancer patients treated with tamoxifen, women suffering from Lynch syndrome or patients with a positive BRCA1 mutation test result), as well as high-risk surgical patients (i.e. with potential complications due to internal co-morbidities).

Human epididymis protein 4 (HE4) is a biochemical marker used in the diagnosis and follow-up of ovarian cancer. Its diagnostic and treatment-predictive value for endometrial cancer is currently being examined. HE4 belongs to a group of proteins known as whey acidic four-disulfide core proteins (WFCD) and exhibits properties similar to trypsin inhibitor [4]. The protein has a molecular weight of 20–25 kDa in advanced glycosylated form and consists of a peptide chain consisting of two WFCD domains [5]. HE4 production has been detected primarily in the epithelium of the distal epididymis and it is assumed that, as a protease inhibitor, it is involved in immune processes and also affects sperm maturation [6, 7]. It has also been established that HE4 is expressed in many tissues, with the highest expression occurring in malignantly transformed epithelial tissues such as ovarian tissue (serous or endometrioid adenocarcinoma), as well as some types of endometrial adenocarcinomas, especially endometrioid and lung cancer [8-10].

Materials and Methods

Two sets of patients were employed in order to examine the role of the HE4 marker in the differential diagnosis of endometrial tumours between July 2009 and June 2014. The first group consisted

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of 150 patients diagnosed with endometrial carcinoma, i.e. its endometrioid subtype. The second group comprised a control group of patients with benign lesions of the endometrium. All 150 patients in this group underwent an endometrial biopsy simultaneously with the members of the first group; in the case of the control group, this procedure excluded the existence of precancerous conditions or malignant findings. Biopsy indications included clinical symptoms (i.e. irregular premenopausal, perimenopausal or postmenopausal bleeding) or suspected ultrasound findings (i.e. suspected endometrial hyperplasia in postmenopausal women or a suspected endometrial polyp). The average age of patients with malignant findings was 65.6 years (40-87) while the average age of women with benign findings stood at 61.8 years (43-81).

Histopathological diagnosis was established on the basis of endometrial curettage or hysteroscopy; diagnosis was followed by blood sampling designed to determine HE4 and CA125 serum levels. Serum samples were separated by centrifugation and immediately processed. In case processing within a 24-hour period was not possible, collected samples were frozen and stored at -80°C until concentration analysis, subsequently performed on an Architect 1000 chemiluminiscent immunoassay analyzer. Exclusion criteria for inclusion in either of the two groups included the presence of other malignant diseases; none of the patients were treated for benign gynecological diseases (endometriosis, pelvic infection, benign ovarian tumor, etc.) and non-gynecological co-morbidities (e.g. liver or autoimmune diseases) associated with elevated CA125 and for benign co-morbidities associated with elevated HE4 (e.g. kidney and liver diseases). All patients included in the malignancy cohort underwent definitive surgical treatment – hysterectomy, bilateral salpingo-oophorectomy, aortopelvic lymphadenectomy, and excisions of suspicious lesions and adhesions of the peritoneal cavity.

Data analysis

In patient groups used in this study, histological findings are always defined by a range of CA125 and HE4 serum levels, assessed according to a median rather than a mean. The median is used to describe selected characteristics instead of an arithmetic mean, especially as the distribution of both biomarkers is not normal (i.e. it is skewed heavily to the right). Using a mean would not be correct in such circumstances as such an approach would be too dependent on extreme values. A logarithmic transformation of HE4 and CA125 levels was carried out for statistical evaluation purposes in order to achieve a normal distribution of both biomarkers. A two-sample *t*-test was used and a *p*-value was determined. Associations with a p-value < 0.05 were considered significant. Logistic regression models developed for subsequent statistical assessment were designed to operate with two identified cut-off values, i.e. highest sensitivity and specificity and a given specificity of 95 %.

Results

The distribution of patients with endometrioid adenocarcinoma according to the FIGO 2009 staging is presented in Table 1.

A low-risk form of the disease, complete with all five prognostic factors (i.e. myometrial invasion < 50%, tumour diameter ≤ two cm, no cervical involvement, tumour grade 1 or 2, and the absence of the extrauterine disease) was established in the case of 73 patients (i.e. 49 %). A high-risk form of the disease where at least one of these factors was absent was established in the case of 77 patients (i.e. 51%).

Table 1. — *Distribution of patients with endometrioid adenocarcinoma including median serum HE4 levels according to FIGO 2009 staging.*

FIGO 2009 Stage	N (%)	Median HE4 (range) (pmol/l)
IA	90 (60.0)	63.0 (29.0−379.0)
IB	26 (17.5)	86.0 (42.0−350.0)
II	10 (6.5)	118.0 (54.0−330.0)
III	17 (11.5)	198.0 (55.0−1014.0)
IV	7 (4.5)	193.0 (68.0−364.0)

FIGO = International Federation of Gynaecology and Obstetrics.

Table 2. — *Median CA125 and HE4 serum levels in relation to histology.*

Histology	N (%)	Median CA125 (range) / IU/ml	Median HE4 (range) / pmol/l
Benign	150	23.0 (5.0−140.0)	45.0 (7.0−185.0)
Malignant (Stage I-II)	126	20.0 (5.0−126.0)	70.0 (29.0−379.0)
Malignant (Stage I-IV)	150	21.0 (5.0−1056.0)	74.0 (29.0−1014.0)

Table 3. — *HE4 serum level in relation to histology – t-test.*

Histology	*p* (HE4)
Benign *vs.* malignant (Stage I-II)	$p < 0.001^*$
Benign *vs.* malignant (Stage I-IV)	$p < 0.001^*$

*statistically significant result.

Table 2 presents the median and range of CA125 and HE4 serum concentrations in three sets: patients with benign lesions, endometrial adenocarcinoma patients suffering from the first two stages of the disease (prevalent both in this study and in general clinical practice), and all patients with malignancies. In the case of CA125, the benign group median was quite paradoxically higher, while an increase in the malignant group median was registered in the case of HE4.

Biomarker serum concentrations were subsequently subjected to statistical analysis in accordance with individual histological findings. In order to compare CA125 and HE4 serum levels between groups of women with malignancies and those with benign tumours, a *t*-test of logarithmic-transformed values of the analyzed markers was performed. A statistically significant difference was established only in the case of HE4 and not in the case of CA125 (Table 3).

A logistic regression model was developed for further evaluation in which CA125 and HE4 were included as independent variables and histological findings were included as the dependent variable. CA125 was found to be insignificant in both models (i.e. benign findings *vs.* all stages of malignancy as well as benign *vs.* malignant Stage I and II findings), and the final models were thus created

Table 4. — *Logistic regression model – predictive value of HE4 in differentiation histology (benign vs. malignant, Stage I–IV).*

Histology comparison (benign *vs.* malignant, Stage I-IV)	Cut off HE4 (pmol/l)	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	AUC (95% CI)
Highest sensitivity and specificity	48.5	87.8 % (80.4−93.2)	56.6 % (46.6−66.2)	81.1 % (73.5−87.8)	68.7 % (64.5−73.4)	76.9 % (71.2−83.3)
Given 95% specificity	99.0	37.4% (28.6−46.9)	93.4% (86.9−97.3)	57.9% (54.8−61.5)	86.0% (76.5−92.9)	76.9% (71.2−83.3)

CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value, AUC = area under curve.

Table 5. — *Logistic regression model – predictive value of HE4 in differentiation histology (benign vs. malignant, Stage I–II).*

Histology comparison (benign *vs.* malignant, Stage I-II)	Cut off HE4 (pmol/l)	Sensitivity (95% CI)	Specificity (95% CI)	NPV (95% CI)	PPV (95% CI)	AUC (95% CI)
Highest sensitivity and specificity	48.5	85.6% (77.0−91.9)	56.6% (46.6−66.2)	81.1% (73.6−87.7)	64.3% (59.8−69.5)	73.6% (67.3−80.7)
Given 95% specificity	99.0	28.9% (20.1−39.0)	93.4% (86.9−93.0)	58.9% (56.1−62.2)	80.0% (67.5−89.7)	73.6% (67.3−80.7)

CI = confidence interval; NPV = negative predictive value; PPV = positive predictive value, AUC = area under curve.

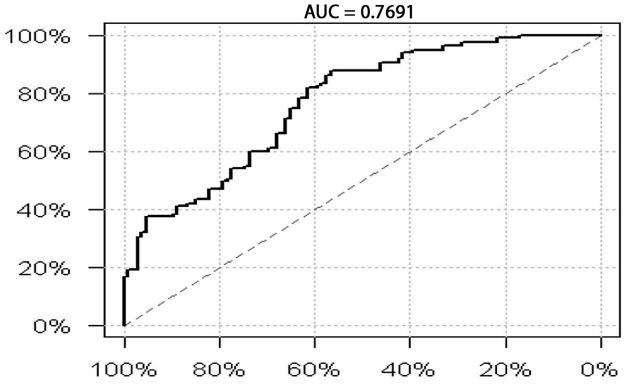

Figure 1. — ROC curve for differentiation between benign and malignant (Stage I–IV) disease.

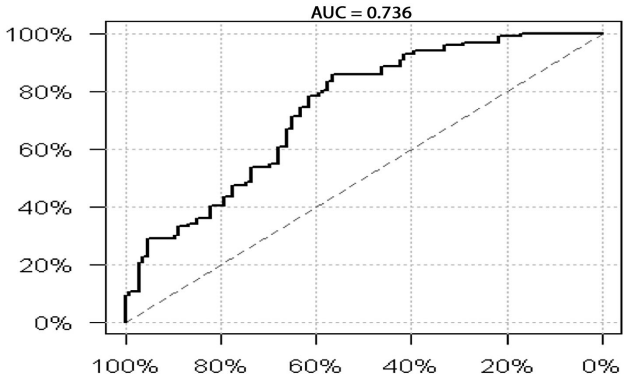

Figure 2. — ROC curve for differentiation between benign and malignant (Stage I–II) disease.

with a single independent variable, i.e. the logarithmic-transformed values of HE4.

Table 4 shows the predictive value of HE4 in the differential diagnosis of endometrial lesions using models for achieving the highest sum of sensitivity and specificity and at a specificity of 95 %; a ROC curve is provided in Figure 1. The model for achieving the highest sum of sensitivity and specificity produced a HE4 cut-off value of 48.5 pmol/l for this differentiation, with a sensitivity of 87.8%, specificity of 56.6%, negative predictive value (NPV) of 81.1%, positive predictive value (PPV) of 68.7%, and AUC of 76.9 %.

Table 5 shows the predictive value of HE4 in the differential diagnosis of benign endometrial lesions and Stage I and II malignancies, likewise using models for achieving the highest sum of sensitivity and specificity and at a specificity of 95%; a ROC curve is provided in Figure 2. The model for achieving the highest sum of sensitivity and specificity produced a HE4 cut-off value of 48.5 pmol/l for this differentiation, with a sensitivity of 85.6%, specificity of 56.6%, NPV of 81.1%, PPV of 64.3%, and AUC of 73.6 %.

Discussion

A number of studies aimed at identifying sensitive biochemical markers useful for the diagnosis and prognosis of patients with endometrial cancer have been published. Although efforts dedicated to the search of new algorithms for the efficient use of biomarkers or a combination thereof are ongoing, it may be said that their clinical benefits remain limited. The object of these studies generally include tumour markers such as antigens – CA125 [11, 12], CA19-9 [13], CEA, and new parameters such as apolipoprotein-1, prealbumin, transferrin [14], prolactin [15], serum amyloid-A [16] or soluble mesothelin related peptides (SMRP) [17].

In the past several years, a range of works have focused on the potential utilization of HE4 in the above context. To some extent, this marker has already established itself in the diagnosis and follow-up of some ovarian cancer histotypes by way of the so-called ROMA (risk of ovarian malignancy algorithm) index, which processes logarithmic-transformed HE4 and CA125 levels and reflects the current status of menopausal patients [18]. The first author to connect the question of clinical utility of HE4 and the issue of endometrial cancer was Moore *et al.* [18] and while subsequent works by others gradually emerged, most were usually limited to an examination of the diagnostic superiority of HE4 in comparison with the more conventional CA125 marker. Most of Moore's studies generally note the elevation of the biomarker for all stages of the disease and point out a greater sensitivity with respect to the detection of its early stages in comparison with CA125. At a specificity of 95%, HE4 sensitivity stands at 45.5% and CA125 sensitivity stands at 24.6%, regardless of disease stage; in Stage I, HE4 exhibits a sensitivity of approximately 17% higher than CA125 [17]. According to Omer *et al.*, who examined HE4 in a group of 64 patients with endometrial cancer and a control group of 60 patients with benign tumours, a cut-off of 59.7 pmol/l provided a sensitivity of 75% at a specificity of 65.5 % [16]. Recent research into HE4 conducted by Angioli *et al.* examined two cut-off values, i.e. 70 and 150 pmol/l; at a specificity of 100%, the achieved sensitivity stood at 59.4% and 35.6%, respectively. In the case of CA125, a cut-off of 35 IU/ml and a specificity of 62.14% resulted in a sensitivity of 19.8 % [19]. A study by Zanotti *et al.* analyzed 195 patients with endometrial cancer and a control group of 125 healthy women. HE4 serum concentrations in patients with malignancies were found to be significantly higher regardless of the stage and grade of the tumour in comparison with healthy controls. In the case of a given specificity of 95%, HE4 exhibited a higher sensitivity regarding the detection of endometrial cancer, namely 66%, while CA125 sensitivity stood at 35% [20].

The present study, which compared two histologically different groups, highlights a significant difference ($p < 0.001$) in median HE4 serum levels, i.e. 45 pmol/l in patients with benign tumours and 74 pmol/l in patients with malignancies. By contrast, CA125 median values (paradoxically slightly higher for women with benign tumours at 23 IU/l and 21 IU/l in the case of patients with malignancies) is statistically insignificant ($p = 0.939$). On logistic regression, CA125 was determined as insignificant in terms of differentiation between benign and malignant lesions, while HE4 – at a cut-off value of 48.5 pmol/l – reached a sensitivity of 87.8%, specificity 56.6%, and negative predictive value of 81.1%, with an AUC value of 0.769 according to the ROC curve. As comparisons of initial stages are prevalent in current clinical practice, this study focused on a comparison of a group of women with benign tumours and patients with endometrial cancer in Stages I and II, employing logistic regression to examine CA125, which also proved to be insignificant in terms of differentiation between benign and malignant lesions, and HE4, which – at a cut-off of 48.5 pmol/l – reached a sensitivity of 85.6% and positive predictive value of 64.3%, with an AUC value of 0.736 according to the ROC curve.

Conclusion

The set of malignancies examined here includes a dominant histological subtype of endometrial cancer while also relatively accurately representing the real clinical presentation of endometrioid adenocarcinoma, including a prevailing representation of patients diagnosed in the early stages of the disease.

This study demonstrated the correlation between HE4 serum levels with malignant histology; moreover, a comparison with CA125 indicated that HE4 is statistically significant with respect to the differential diagnosis of benign and malignant lesions of the endometrium.

The diagnostic benefits of HE4 could be considered especially in the case of patients at increased risk of endometrial cancer as well as in the case of patients with serious internal co-morbidities. In combination with clinical and ultrasound findings, HE4 could help with the differentiation of prognostically varied patient groups as well as with the decision-making process associated with the development of individual treatment plans.

However, the optimal cut-off for HE4 has not been established yet and further studies are needed. The results of more a extensive study could establish whether or not it is possible to implement HE4 analysis in routine clinical practice in the future, hopefully with clear benefits for patient diagnosis, prognosis, and follow-up.

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Address reprint requests to:
L. MINAR, M.D., Ph.D.
Department of Obstetrics and Gynaecology
Faculty of Medicine, Masaryk University Brno
and University Hospital Brno
Obilni trh 11
602 00 Brno (Czech Republic)
e-mail: lubosminar@seznam.cz