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HE4 is superior to CA125 in the detection of recurrent disease in high-risk endometrial cancer patients

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Abstract

Objective: To date, biomarkers are not routinely used in endometrial cancer diagnosis, prognosis, and follow-up. The purpose of this study was to evaluate whether serum HE4 was related to clinicopathological risk factors and outcome. Second, the role of serum HE4 and CA125 was assessed as indicator for recurrent disease during follow-up.

Methods: A total of 174 patients with endometrial cancer between 1999 and 2009 were selected for this retrospective study. Serum HE4 and CA125 were analyzed at primary diagnosis, during follow-up, and at the time of recurrence. Correlations with clinicopathological factors were studied by univariate and multivariate survival analyses. Lead time was calculated in order to determine which serum marker was elevated prior to clinical detection of recurrent disease.

Results: Serum levels of HE4 and CA125 were significantly associated with high tumor grade, myometrial invasion, lymph node involvement, and advanced stage ($p < 0.01$). HE4 was an independent prognostic factor for reduced disease-free survival and overall survival with hazard ratios of 2.96 (95% confidence interval: 1.18–7.99) and 3.27 (95% confidence interval: 1.18–9.02), respectively. At recurrence, 75% of the patients had an elevated HE4 compared to 54% with an elevated CA125. HE4 levels were more frequently elevated in patients with distant metastasis compared to local recurrences, 67% and 37%, respectively. Serum HE4 detected a recurrence with a median of 126 days earlier than clinical confirmation.

Conclusion: Elevated serum HE4 is an independent risk factor for reduced disease-free survival and overall survival. HE4 seems to be superior to CA125 in the detection of recurrent disease during follow-up, mainly in high-risk endometrial cancer patients who are more prone to distant metastasis.

Keywords

Endometrial cancer, recurrence, HE4, CA125, biomarker

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Introduction

Endometrial cancer (EC) is the most common malignancy of the female genital tract with an increasing incidence.[1] Since most patients present with symptoms of postmenopausal or abnormal uterine bleeding, 80% of the EC patients are diagnosed at an early stage and have a favorable prognosis with a 5-year survival rate around 80%–90%.[2] Yet, 13%–17% of the EC patients develop recurrent disease, mostly within 3 years of primary treatment.[3] In case of an isolated recurrence at

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the vaginal vault, local treatment is often with curative intent. For distant metastasis, treatment options are limited and prognosis is poor.[2,4,5]

To date, follow-up consists of monitoring clinical symptoms combined with gynecological examination, yet it is known that 41%–83% of the recurrences are symptomatic.[4,5] In general, there is no consensus about surveillance strategies. In patients with low-risk EC, the added value of intensive surveillance has not been demonstrated.[4,5] Also for patients with high-risk EC, with a significant risk of recurrence, it is not clear whether intensive surveillance contributes to early detection and/or an improved outcome. In addition to clinical examination, cytology of the vaginal vault and chest X-ray has been evaluated for the detection of recurrent disease, both with a low detection rate.[3]

Imaging techniques such as ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET) can be used in symptomatic patients to assess the extent of the disease. Yet, routine use is not advocated because of the low detection rate for recurrent disease and the fact that imaging did not improve survival when compared to clinical examination.[3,5,6] Adding biomarkers to routine follow-up might be beneficial; although for CA125, one of the most studied biomarkers in EC, the value seems limited.[7,8] Several studies have demonstrated the correlation of elevated CA125 with advanced-stage disease and lymph node metastasis.[9,10] However, the sensitivity and specificity are poor, and therefore, CA125 is not routinely used in clinical practice in the diagnostic work-up of EC.[7,8,11] Recent studies suggest a promising role for human epididymis protein 4 (HE4) in the diagnostic work-up and follow-up of patients with EC.[9,12–14] The Australian National Endometrial Cancer Study (ANECS) is the largest prospective study to date which showed that HE4 was correlated with clinicopathological factors and demonstrated that HE4 was an independent predictor of recurrence-free survival.[15]

HE4 belongs to the protease inhibitors and is localized in the epithelial cells of the epididymal duct. However, expression also has been reported in a number of tissues outside the male reproductive system, as well as in various types of carcinomas, including EC.[9,16,17] It is presumed that HE4 plays a role in natural immunity, yet little is known about its potential role in carcinogenesis.[18]

There is accumulating evidence that HE4 is superior to CA125 in the diagnostic work-up of EC in terms of a higher sensitivity and specificity.[9,19,20] To date, little is known about the predictive value of HE4 and CA125 to detect a recurrence. Angioli et al. reported that preoperative HE4 levels were higher in patients who developed a recurrence compared to patients without recurrence. These data support the potential role of HE4 in both the identification of high-risk patients and in the detection of a recurrence.[14,20] Brennan et al. also showed that HE4 was a sensitive and specific predictor of recurrent disease, particularly in patients with endometrioid histology with an area under the curve (AUC) of 0.87 compared to 0.67 for CA125.[13]

In a small series of ovarian cancer patients (n = 23), HE4 was superior to CA125 in the detection of a recurrence. In addition, increased serum levels of HE4 preceded the rise of CA125 by 4.5 months.[21] The aim of our study was to compare the dynamic changes of serial serum measurements of HE4 and CA125 in relation to recurrent disease in EC patients.

Material and methods

Patients

A total of 174 patients who underwent primary surgical treatment for EC at the Radboud University Medical Center (Nijmegen) between 1999 and 2009 and from whom preoperative serum was stored were selected for this retrospective study. All endometrial histological subtypes were included, whereas patients with progressive disease were excluded.

Preoperative diagnosis was based on dilatation and curettage (D&C) or Pipelle endometrial sampling. All patients underwent at least an abdominal hysterectomy and bilateral salpingo-oophorectomy. Surgical staging with pelvic and/or para-aortic lymphadenectomy was performed in patients with preoperative high tumor grade. All patients were re-staged according the International Federation of Gynecology and Obstetrics (FIGO).[22] Adjuvant radiotherapy was applied according to the postoperative radiation therapy in endometrial carcinoma (PORTEC) criteria (deep myometrial invasion (MI), high tumor grade, and age >60 years) for treatment of stage I–II patients, and chemotherapy was administered in patients with stage III–IV disease. Medical records of the patients were carefully reviewed.[23]

Patients' follow-up was performed from the date of primary treatment until the last visit or death. For the first 3 years, patients were seen every 3 months and every 6 months afterwards till 5 years after diagnosis. After 5 years of follow-up, patients were dismissed.

Recurrent disease was detected by radiological imaging or confirmed by histological biopsy. The disease-free interval had to be at least 3 months after primary treatment.[24,25]

The study was approved by the Ethics Committee of the Radboud University Medical Center (Nijmegen).

Aims of the study

The primary aim of this study was to evaluate whether serum HE4 and CA125 were related to clinicopathological risk factors and outcome. Secondary aim was to

compare serum HE4 and CA125 levels at the time of recurrence and to assess their role as indicator of recurrent disease in serial serum measurements.

Serum storage

Blood samples were collected in dry tubes by vena puncture and centrifuged at 2000*g* for 10 min. Serum was stored at −40°C until analyzed.

HE4 and CA125 measurements

HE4 and CA125 serum levels were measured by the LUMIPULSE chemiluminescent enzyme immunoassay (CLEIA) kit according to the manufacturer's (Fujirebio Diagnostic, Inc., Malvern, PA, USA) instructions.[26]

Serum samples of four different time points were used: (1) at primary diagnosis, (2) post-surgery, (3) follow-up, and (4) at recurrence.

Statistical analysis

Statistical analysis was performed using GraphPad prism version 5.3 (GraphPad Software, Inc., La Jolla, CA, USA). In all tests, $p < 0.05$ (two-sided) was considered to indicate statistical significance.

Serum concentrations of HE4 and CA125 were presented as median values, and log transformation was applied because the distribution was positively skewed. Clinical and pathological parameters of the non-recurrence and recurrence groups were compared using the Pearson χ^2 or Fisher exact test for categorical variables, also using the independent t-test or Mann–Whitney-U test when appropriate.

Paired serum measurements were compared with the Wilcoxon signed-rank test. The Cox-proportional hazard model was used to assess the prognostic value of serums HE4 and CA125 as log transformed continuous factors, both in univariate and multivariate analyses. Traditional prognostic factors such as FIGO stage, age, tumor grade, MI, and lymphovascular space invasion (LVSI) were included in a base model. HE4 and CA125 were entered separately in a second block. Points estimated were reported as hazard ratios (HRs) and 95% confidence intervals (CIs). Based on published data, the used cut-off value for HE4 was 70 pmol/L and for CA125 was 35 U/mL.[27–30]

Recurrent disease was detected by radiological imaging or confirmed by histological biopsy. The disease-free period had to be at least 3 months after primary treatment. The time to recurrence was noted, and subsequently, the time between the rise of serum levels of HE4 and CA125 and recurrence was calculated. The time between the elevation of serum markers and the recurrence was defined as the lead time to recurrence. The lead time (days) was calculated for both HE4 and CA125.[25]

Results

Patients

A total of 174 patients with EC with available preoperative serum sample were analyzed. After review, 17 cases were excluded because of progressive disease after primary diagnosis, resulting in 157 patients that could be included in our study.

Baseline patients' characteristics are demonstrated in Table 1. Patients had a median age of 63 years and a body mass index (BMI) of 27 kg/m^2. In all, 48 (31%) patients developed a recurrence, of which 40% (n = 19) was located in the pelvis (loco regional) and 60% (n = 29) outside the pelvis (distant recurrences). Patients who developed recurrent disease were more likely to have

Table 1. Baseline patient characteristics.

	N = 157	%
Age (years; median)	63 (56–71)	
BMI (kg/m^2; median)	27 (23–33)	
Histology		
Endometrioid	122	78
Non-endometrioid	35	22
FIGO		
IA	17	10
IB	83	53
II	11	7
III	27	17
IV	19	13
Grade		
I	27	17
II	71	45
III	59	38
LVSI		
Yes	62	39
No	64	41
Missing	31	20
MI		
<50%	74	48
≥50%	78	50
Missing	5	2
Lymph node involvement		
Yes	13	25
No	39	75
Not assessed	105	
Recurrence		
Locoregional	19	40
Distant	29	60
None	109	
Time to recurrence (median)		
Locoregional	8 (5–18)	
Distant	15 (5–30)	
Overall survival (median)		
Non-recurrence	67 (50–104)	
Recurrence	24 (9–48)	

LVSI: lymphovascular space invasion; MI: myometrial invasion; BMI: body mass index; FIGO: International Federation of Gynecology and Obstetrics.

Data are presented as numbers with percentage or as median with range.

Table 2. Clinicopathological factors in relation to serum levels of HE4 and CA125.

	HE4	p value	CA125	p value
Age (years; median)				
<60	60 (40–94)	<0.01	15 (8–36)	0.28
≥60	90 (56–160)		17 (10–42)	
BMI (kg/m^2; median)				
<25	75 (38–112)	0.45	16 (9–33)	0.90
≥25	73 (50–134)		16 (9–43)	
Histology				
Endometrioid	73 (46–124)	0.21	14 (7–27)	<0.01
Non-endometrioid	79 (50–169)		28 (15–72)	
FIGO				
I–II	62 (45–101)	0.01	12 (8–22)	<0.01
III–IV	79 (51–153)		17 (9–44)	
Grade				
I–II	69 (44–112)	0.05	13 (7–25)	<0.01
III	81 (50–164)		25 (12–62)	
LVSI				
Yes	96 (57–169)	<0.01	23 (11–66)	<0.01
No	59 (37–86)		12 (8–21)	
MI				
<50%	59 (41–97)	<0.01	12 (8–20)	<0.01
≥50%	86 (57–156)		25 (14–57)	
Lymph node involvement				
Yes	153 (90–260)	<0.01	41 (19–194)	0.03
No	68 (46–96)		19 (11–43)	

LVSI: lymphovascular space invasion; MI: myometrial invasion; BMI: body mass index; FIGO: International Federation of Gynecology and Obstetrics. Data are presented as medians with 25–75^e percentile. HE4 (pmol/L) and CA125 (U/mL).

FIGO stage III/IV disease, high tumor grade, and LVSI (data not shown). There were no significant differences in age, BMI, or tumor type between patients experienced a recurrence and those who remained disease free. The median overall survival (OS) of patients with recurrence was 24 months (9–48 months) and without recurrence was 67 months (50–104 months). Locoregional recurrences developed after a median of 8 months, whereas distant recurrence developed after a median of 15 months; however, this was not significantly different.

Serum HE4 and CA125 in relation to clinicopathological risk factors and outcome

In Table 2, clinicopathological factors in relation to serum levels HE4 and CA125 are shown. Both serum markers HE4 and CA125 were significantly related to clinicopathological risk factors, such as FIGO stage, LVSI, MI, and lymph node involvement.

HE4 and CA125 levels were higher in stage III/IV disease, in the presence of LVSI, MI ≥50%, and lymph node involvement. CA125 was significantly higher in grade 3 and non-endometrioid histology, whereas HE4 levels were higher in patients ≥60 years. BMI was not related with either HE4 or CA125 serum levels. Unfortunately, we were not able to differentiate different EC stages with serum HE4. However, HE4 has an AUC of 0.72 to predict the involvement of lymph nodes. Based on the receiver operating characteristic curve (ROC) curve (Supplementary Figure 1), an HE4 cut-off of 130 mmol/L reported the best performance in terms of a sensitivity (65%) and a specificity (79%) to correctly classify patients with positive lymph nodes.

HE4 and CA125 in relation to recurrence

Serum levels in relation to recurrence are shown in Table 3, and serial measurements of HE4 and CA125 are illustrated in Figures 1 and 2. Preoperative serum

Table 3. HE4 and CA125 levels in relation to recurrence.

	Non-recurrence	Recurrence	p value
	N = 108	N = 42	
Median HE4			
Diagnosis	68 (46–110)	86 (51–199)	0.015
Post-surgery	43 (32–66)	75 (30–152)	0.021
Follow-up	41 (29–75)	138 (59–340)	
Recurrence	N/A	235 (56–570)	<0.001
Median CA125			
Diagnosis	14 (8–27)	22 (10–83)	0.023
Post-surgery	8 (5–11)	22 (12–52)	<0.001
Follow-up	7 (5–10)	37 (15–114)	
Recurrence	N/A	68 (16–221)	<0.001

Data are presented as medians with 25–75^e percentile. HE4 (pmol/L) and CA125 (U/mL).

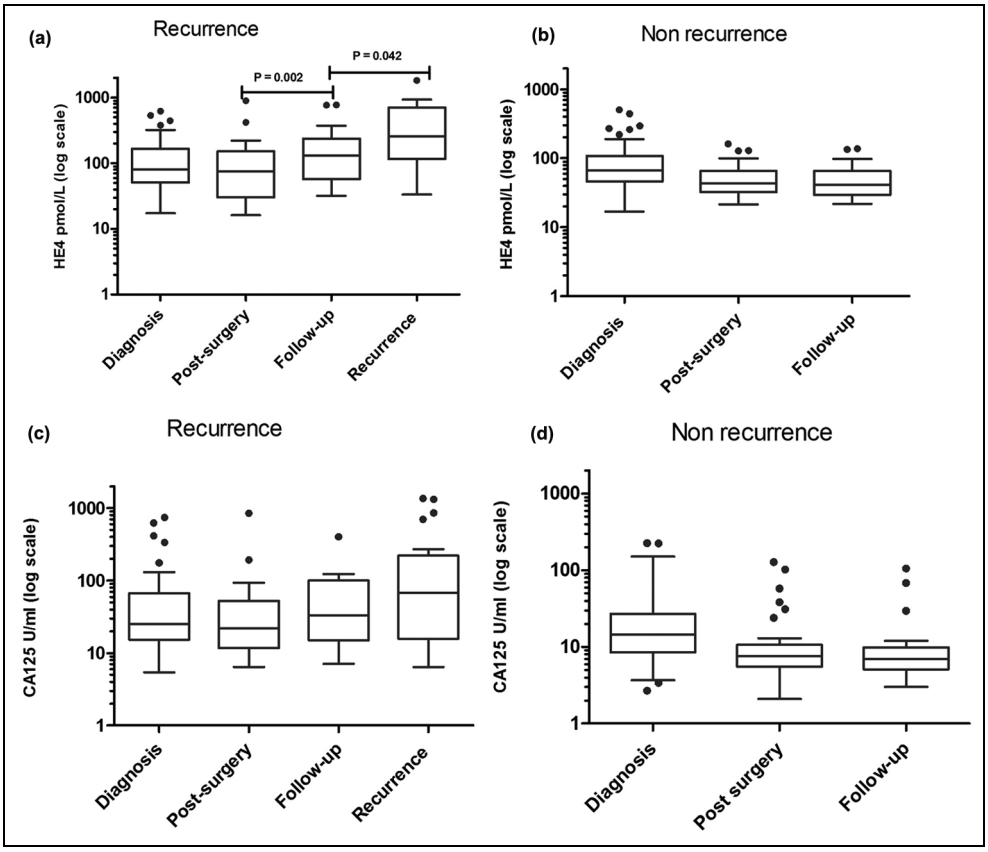

Figure 1. HE4 and CA125 in recurrent and non-recurrent patients with EC. Box plots display serum levels at different measure points, demonstrating medians with interquartile range. HE4 levels of (a) patients with recurrence and (b) patients without recurrence. CA125 levels of (c) patients with recurrence and (d) without recurrence.

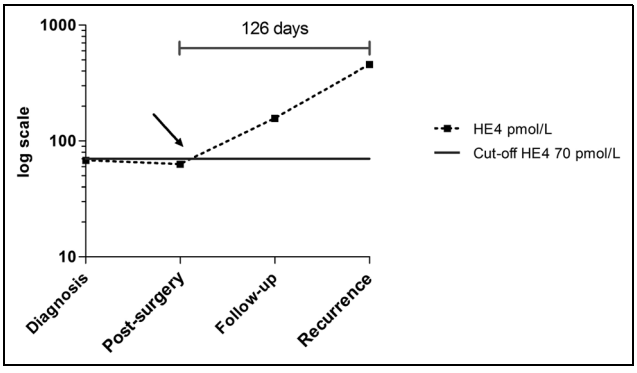

Figure 2. Lead time of HE4. HE4 median values are depicted for each time point (diagnosis, post-surgery, follow-up, and recurrence). The arrow indicates the point where HE4 crosses the cut-off levels with a median of 126 days before clinical detection of recurrent disease.

levels of both HE4 and CA125 were significantly higher in patients who experienced a recurrence than those who remained disease free. HE4 levels were 86 and 63 pmol/L ($p = 0.01$) and 75 and 14 U/mL ($p = 0.02$) for CA125, respectively.

In patients with recurrent disease, 75% (n = 18) had an elevated HE4 compared to 54% (n = 13) with an elevated CA125 (data not shown). With respect to the location of a recurrence, only 39% (n = 7) of the patients with a local recurrence had elevated HE4 values compared to 16% (n = 3) with an elevated CA125. In patients with distant metastasis, elevated HE4 levels were present in 67% (n = 20) where only 55% (n = 16) had an elevated CA125 (data not shown).

Disease-free survival (DFS) was significantly associated with elevated HE4 and CA125 in the univariate analysis. HRs were 4.23 ($p < 0.001$) and 3.16 ($p < 0.001$), respectively (Table 4).

Both HE4 and CA125 were significantly associated with OS in the univariate analysis with HRs of 7.77 ($p < 0.001$) and 3.41 ($p < 0.001$), respectively.

Next to FIGO stage and age, HE4 showed to be the strongest independent prognostic factor for OS followed by CA125 with HR = 3.27 ($p = 0.02$) and HR = 2.14 ($p = 0.03$), respectively.

With respect to serial measurements, we used four different time points; median time between primary diagnosis and post-surgery was 53 days (21–114 days) and 55 days (16–92 days) between follow-up and

Table 4. Hazard ratios of disease-free survival and overall survival.

Disease-free survival						
	Univariate			Multivariate		
	HR	95% CI	p value	HR	95% CI	p value
Base model[a]						
FIGO stage III–IV versus I–II	**4.75**	**2.63–8.60**	**<0.01**	**3.63**	**1.84–7.15**	**<0.01**
Age (years) >60 versus ⩽60	**2.15**	**1.16–3.99**	**0.01**	**2.36**	**1.17–4.76**	**0.01**
Grade III versus I–II	**2.30**	**1.28–4.11**	**<0.01**	1.63	0.84–3.16	0.15
MI >50% versus ⩽50%	1.53	0.81–2.87	0.18	0.99	0.50–1.98	0.98
LVSI Yes versus no	**2.36**	**1.23–4.52**	**<0.01**	1.18	0.58–2.41	0.65
Additions to model (all continuous, log transformed, and separately entered)						
Serum HE4	**4.23**	**2.02–8.82**	**<0.01**	**2.96**	**1.18–7.94**	**0.03**
Serum CA125	**3.16**	**1.95–5.12**	**<0.01**	1.58	0.86–2.91	0.14
Overall survival						
	Univariate			Multivariate		
	HR	95% CI	p value	HR	95% CI	p value
Base model[a]						
FIGO stage III–IV versus I–II	**5.75**	**3.19–10.34**	**<0.01**	**3.69**	**1.86–7.31**	**<0.01**
Age (years) >60 versus ⩽60	**2.19**	**1.19–4.06**	**0.01**	**2.37**	**1.18–4.78**	**0.02**
Grade III versus I–II	**3.42**	**1.92–6.10**	**<0.01**	1.61	0.83–3.13	0.15
MI >50% versus ⩽50%	**2.57**	**1.29–5.09**	**<0.01**	1.01	0.51–2.02	0.96
LVSI Yes versus no	**2.08**	**1.10–3.94**	**0.02**	1.15	0.56–2.40	0.69
Additions to model (all continuous, log transformed, and separately entered)						
Serum HE4	**7.77**	**4.00–15.07**	**<0.01**	**3.27**	**1.18–9.02**	**0.02**
Serum CA125	**3.41**	**1.99–5.82**	**<0.01**	**2.14**	**1.09–4.20**	**0.03**

MI: myometrial invasion; LVSI: lymphovascular space invasion; BMI: body mass index; FIGO: International Federation of Gynecology and Obstetrics; CI: confidence interval.

[a]The base model consisted of traditional prognostic factors, and we separately entered the parameters in a second block. The multivariate analysis showed that besides FIGO stage and age, only HE4 turned out to be an independent prognostic factor for DFS with an HR of 2.96 (p = 0.03).
Bold values indicate the significant HR's.

recurrence. At all serial measurements, serum levels of both HE4 and CA125 were significantly higher in patients who developed a recurrence compared to those without recurrence. As illustrated in Figure 1, serum levels of HE4 and CA125 remained low in the non-recurrence group.

Figure 2 displays that serum levels of HE4 were significantly increased at the follow-up moment prior to the clinical detection of recurrent disease ($p = 0.042$). In contrast, increase in serum levels of CA125 was minor and not significant.

The lead time for HE4 was calculated to determine the time between elevation of the serum marker and clinical detection of recurrent disease. Figure 2 displays the median HE4 levels at the different serial measurements.

The rise of HE4 above the used cut-off value detected a recurrence with a median of 126 days earlier than the clinical confirmation of recurrent disease (indicated with black arrow in Figure 2).

Discussion

In this study, we showed that elevated serum levels of HE4 and CA125 correlate with well-established prognostic factors for EC, such as high tumor grade, MI, lymph node involvement, LVSI, and advanced stage and that elevated serum levels correlate with DFS and

OS. During follow-up, serum levels of HE4 were superior to CA125 with respect to identification of patients with recurrence as well as the prediction of a recurrence. Preoperative measurement of serum HE4 levels is superior to CA125 and could help to identify patients at risk of advanced-stage disease, lymph node involvement, and recurrence of disease. This might contribute to a more personalized treatment and follow-up plan.

The results of our study showed that HE4 correlates with well-known prognostic factors and are in line with previous studies.[9,10,12,15,20,28,31,32] Serum HE4 was correlated with FIGO stage, tumor grade, MI, and LVSI. Although our cohort is relatively old in comparison with other studies, cohort size was fairly similar. Besides a correlation between HE4 and risk factors for lymph node involvement, we found a direct correlation between serum HE4 and the presence of lymph node metastasis. However, results about lymph node involvement and HE4 are still conflicting. A possible explanation could be that other studies report low numbers of performed lymphadenectomy, whereas median HE4 levels may not reach significance in patients with lymph node involvement.[9,12,32] In agreement with other studies, we did not observe a correlation between HE4 and the different histological subtypes, but the number of non-endometrioid EC in our cohort was relatively small to draw final conclusion.[20,32] Outcome in patients with EC is mainly determined by clinicopathological factors. As expected, clinicopathological factors were significantly associated with OS in the univariate analysis providing the validity of our patient cohort. Yet, in this study, we observed that elevated HE4 was an independent prognostic factor for both DFS and OS in patients with EC which is in line with the recent studies by Stiekema et al.[32] and Mutz-Dehbalaie et al.[33] The ANECS confirmed these results in a prospective study among 373 patients with EC. They showed that HE4 levels were higher in patients with an advanced FIGO stage, and it was a better predictor of outer-half MI than CA125. Besides this, it was shown that HE4 was an independent predictor of recurrence-free survival. To date, this is one of the few studies which demonstrate the value of HE4 as an independent prognostic factor for both DFS and OS in patients with EC.

With respect to the comparison of HE4 and CA125 in relation to recurrent disease, we showed that HE4 was superior to CA125 in the detection of recurrent disease. These findings are in accordance with previously published studies.[13,14] With comparable cut-off values, Brennan et al. found an elevated HE4 in 80% of the recurrences, whereas CA125 was elevated in only 47%. Yet, those 26 patients with recurrent disease in this study cohort (n = 98) consisted mainly of patients with local recurrence (n = 20) and only few (n = 6) with distant recurrences. On the contrary, our cohort contained mainly distant recurrences (60%) where median HE4 values of distant recurrences were borderline significant compared to serum values of local recurrences ($p = 0.058$).[27,28]

HE4 concentrations at time of the recurrence were higher than at primary diagnosis. This finding is conflicting with literature.[13,14] Angioli et al. and Brennan et al. found no significant difference in HE4 concentration at primary diagnosis and recurrence. This might be explained by the difference in study populations; as outlined above, our cohort contained less local recurrences and more distant recurrences. A possible explanation is that HE4 levels are associated with an increased metastasis-associated tumor burden. The relationship of HE4 concentration and tumor burden is described in patients with ovarian cancer where HE4 levels were correlated with the presence of tumor burden after optimal and suboptimal cytoreductive surgery.[34] However, so far, data about the relationship of HE4 serum levels and tumor burden in EC patients are still lacking.

Serum levels of HE4 detected a recurrence with a median of 126 days before clinical confirmation which implies that HE4 is a more sensitive and useful tumor marker for detecting a recurrence during follow-up than CA125. HE4 might be more specifically related to the amount of tumor tissue when compared to CA125. This finding is supported by a study conducted in ovarian cancer patients where HE4 detected a recurrence 4.5 months earlier than CA125.[21] Patients who developed recurrent disease, especially those with distant recurrences, were more likely to have an advanced FIGO stage, a high tumor grade, and LVSI, which is in concordance with literature.[35–37] Our study shows that high-risk patients are prone to develop distant metastasis, and therefore, we suggest that their follow-up could be improved by serial measurements of HE4 to detect early recurrent disease. Whether earlier detection of recurrent disease will contribute to an improved outcome needs to be determined.

This study is the first to analyze dynamic changes of HE4 during the follow-up period of EC patients. All serum samples were managed in a standardized fashion. Histology was performed by an expert gynecologic pathologist. However, some limitations need to be addressed. Serum of patients was stored over a long timeframe and possible influences on the HE4 protein cannot be ruled out. However, repeated measurements of CA125 remained stable after years of storage and did not significantly change. The selection of our study cohort was based on the availability of consecutive serum samples, and hence, this might result in selection bias of our study population. In this study, we used cut-off values of 70 pmol/L and 35 U/mL for HE4 and CA125, respectively. However, the cut-off level of 35 U/mL for CA125 defines normal and pathological serum levels for ovarian cancer, but for EC patients, this has not been identified yet.[29] Besides this, there is

still an ongoing debate about the best cut-off for HE4 in the diagnostic work-up of EC. Cut-off levels of HE4 vary from 60 to 100 pmol/L, but most studies used 70 mmol/L because it yields the best sensitivity and specificity.[20,28,30] In accordance with the analysis of Capriglione et al.,[31] we performed an ROC analysis of HE4 and CA125 for the prediction of tumor stage. Unfortunately, we could not confirm these findings neither for HE4 nor for CA125. This study reported HE4 cut-off levels for different tumor stages with high sensitivity and specificity values. This could be explained by the fact that our study cohort is different from the study cohort of Capriglione et al. with more patients with advanced-stage disease (30% vs 21%, respectively) and more non-EC patients (22% vs 6%, respectively). Furthermore, cut-off levels for detecting recurrent disease have not been identified yet and should be investigated in future research.

A future prospective study on a larger cohort of patients with EC is needed to affirm the preoperative value of serum HE4 and the use of HE4 in routine follow-up. Preoperative staging of patients with EC remains challenging, and the promising data of HE4 could contribute to a more personalized approach in EC patients. Currently, tumor histology, tumor grade, MI, LVSI, and age are used to tailor treatment. However, most of the prognostic risk factors are based on final pathology, and therefore, an EC algorithm for the identification of high-risk EC, based on preoperative data, is urgently needed. The risk of endometrial malignancy (REM) scoring system has recently been validated to discriminate between benign disease and EC with a high sensitivity and specificity. In addition, this REM can differentiate between low- and high-risk EC patients and hence contribute to tailor treatment.[38,39] Also imaging techniques combined with HE4 are used to assess the risk of MI, cervical involvement, and lymph node involvement preoperatively in order to modulate surgery.[40] Interestingly, we also found that HE4 significantly contributes to the prediction of lymph node involvement with an AUC of 0.72 and a sensitivity of 65% and specificity of 79% (cut-off value: 130 mmol/L). Because there is an ongoing debate about the therapeutic role of a lymphadenectomy, HE4 could identify those patients who are at great risk of lymph node involvement and helps planning the required surgery. However, HE4 serum levels alone or in combination with other clinical features, histological features, or imaging techniques could lead to a preoperative algorithm to avoid under or overtreatment and sending patients to specialized centers if necessary.

In conclusion, HE4 is correlated with histological prognostic factors in EC and is a strong independent prognostic factor for DFS and OS. Furthermore, HE4 shows to be superior in the detection of recurrent disease during the follow-up period compared to CA125, especially in high-risk EC developing distant metastasis. Serum HE4 contributes to the preoperative identification of low- and high-risk patients and could therefore contribute to a more personalized treatment and follow-up plan.

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Declaration of conflicting interests

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Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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Informed consent

The study was approved by the Ethics Committee of the Radboud University Medical Center (Nijmegen) and informed consent was given.

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