RESEARCH ARTICLE

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Human Epididymis Protein 4 (HE4) and Cancer Antigen 125 (CA125) for Prediction of Optimal Primary Surgery in Non-Mucinous Epithelial Ovarian Cancer

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Abstract

Objective: To determine the relationship between pre-operative HE4 and CA125 levels in non-mucinous epithelial ovarian cancer cases (EOC) and outcomes of primary surgery for prediction of optimal surgery. **Methods:** A retrospective study was performed on non-mucinous EOC who underwent primary surgery at King Chulalongkorn Memorial Hospital from 2016 to 2020. Demographic and clinical characters were collected. Histopathology and pre-operative tumor markers namely HE4 and CA125 were also recruited. Primary surgical outcomes were classified as optimal (OS) and suboptimal surgery (SS). **Results:** One hundred and seventy patients were enrolled in the study. There were 130 and 40 cases in OS and SS, respectively. Average age and body mass index (BMI) of EOC were 54.2 years old and 23.1 Kg/m2, respectively. Both groups had comparable demographic characteristics. Two-thirds (103/170) and one-third (63/170) had early stage and clear cell histopathology, respectively. The median level of HE4 were 118.60 and 603.45 pmol/L in OS and SS, respectively. OS and SS had average CA125 at 146.95 and 814.70 U/L, respectively. The best cut-off point of HE4 and CA125 less than 170.95 pmol/L and 316.4 U/mL gave predicting OS with area under curve (AUC) at 0.78 and 0.75, respectively. HE4 and CA125 cut-off point had sensitivity, specificity, positive predict value (PPV) and negative predictive value (NPV) at percentage of 60.8/60.8, 87.5/82.5, 94.1/91.9 and 40.7/39.3, respectively. **Conclusion:** HE4 and CA125 of non-mucinous EOC among OS had significantly less than SS and could be the predicting of optimal surgery.

Keywords: Non-mucinous epithelial ovarian cancer- HE4- optimal primary surgery- prediction

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Introduction

Although, ovarian cancer (OC) ranks the third in prevalence, but the mortality rate of OC is still high [1]. Every year, OC affects over 200,000 women across the world [2]. The age-standardized incidence rate (ASR) of ovarian cancer in Thai women has increased from 6.2 to 7.9 per 100,000 Thai female population in the past two years [3].

Majority of ovarian cancer is epithelial ovarian cancer (EOC). The current standard treatment of EOC is maximal primary cytoreductive surgery followed by chemotherapy [2]. The goal for surgery is optimal surgery (OS), regarded as being able to completely remove the majority of the tumor leaving only residual tumor lesions measuring less than or equal to one centimeter in maximal diameter. OS is the most important prognostic factor for both overall survival and progression-free survival [2]. In the intergroup studies AGO-OVAR 3, 5 and 7, they were found that those who have no residual disease had better prognosis in terms of recurrence and mortality rate [2]. In case that OS cannot be achieved in the first operation, neoadjuvant chemotherapy (NACT) may be considered. Prediction for the chance of achieving optimal surgical outcome is important for judging the appropriate management.

For maximizing benefits and reducing complications from surgery, pre-operative factors namely demographics, staging of disease, laboratory results, histopathology and radiologic imaging were the topic of interest. Despite many recent studies having focused on evaluation of eligible patients who achieved OS, there are no established accurate or reliable pre-operative parameters to predict surgical outcome. Attempts have been made using non-invasive biomarkers, imaging predictive scores and invasive procedures to predict outcome of primary surgery. But systematic review shows that the CT-based model has low sensitivity and specificity for prediction of primary surgical outcome [2]. In 2006, the Fagotti scoring system was developed and used diagnostic laparoscopy to predict

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likelihood of optimal surgical outcome in OC, showing a 100% positive predictive value [4, 2]. However, due to limitations in terms of surgical equipment, availability of laparoscopic surgeons and socioeconomic barriers in Thailand and other similar developing countries, radiologic imaging or diagnostic laparoscopy may not be accessible for all pre-operative patients. Hence in such settings, non-invasive predictive factors such as age, body mass index (BMI), performance status and laboratory results may be used to preoperatively predict surgical outcome in EOC.

Cancer Antigen 125 (CA125) is a serum glycoprotein produced by many tissues of the body and its elevation can be found in both normal conditions and in some certain diseases. Elevated levels of CA125 can be found in physiologic conditions such as menstruation and pregnancy. Pathologic conditions which have elevated levels of CA125 include endometriosis, intraabdominal inflammation and EOC [1]. However, CA125 has a low specificity for EOC, particularly in mucinous types. Human epididymis protein 4 (HE4) is a serum glycoprotein which has a marked elevation in EOC patients, especially in non-mucinous types [1]. Currently, there are many studies which determine the association of these laboratory values with surgical outcome of primary surgery in EOC.

Therefore, the primary objective of this study was to determine the relationship between pre-operative HE4 levels in non-mucinous EOC and surgical outcome. The secondary objectives were to determine the differences in pre-operative CA125 levels and surgical outcome as well as determine cut-off points for both CA125 and HE4 in prediction of surgical outcome in order to guide management for non-mucinous EOC.

Materials and Methods

This retrospective study was performed at the Department of Obstetrics and Gynecology, King Chulalongkorn Memorial Hospital (KCMH), Bangkok, Thailand. This study was approved by the Ethic Committee of Chulalongkorn University (IRB No. 890/64). Patient records of those who were diagnosed with non-mucinous EOC between January 2016 and December 2020 inclusive were collected. Non-mucinous EOC patients who underwent primary surgery and had pre-operative serum HE4 and CA125 levels were included in this study. Those who had incomplete medical data and had other primary malignancies were excluded.

Data reviewed from medical records included age at the time of diagnosis, BMI, underlying diseases, pre-treatment laboratory investigations (CA125 and HE4 levels, ROMA score, complete blood count and serum albumin), tumor features (FIGO stage, histological subtype, grading), operative findings (operative time, estimated blood loss (EBL), ascites, extensive surgery such as bowel, bladder and vascular surgery, residual tumor after surgery and complications) and surgical outcomes namely OS or suboptimal surgery (SS).

Statistical analysis was done by SPSS Version 29 (SPSS Inc, Chicago, IL, USA). Demographic data were analyzed using descriptive statistics. Median, mean and standard deviation (SD) were used for continuous data. Mann-Whitney U test and Chi-squared test were used for categorical data. The appropriate cut-points for HE4 and CA125 were determined using an receiver operating curve (ROC). Accuracy of the aforementioned cut-off points were calculated using sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). A p-value of less than 0.05 (<0.05) was considered statistically significant.

Two independent means formula was used for sample size calculation. Alpha and beta error were set at level of 0.05 and 0.2, respectively. According to Feng's study in year 2020, at least one hundred cases were required for appropriate sample size.

Results

From January 2016 to December 2020, history of 664 OC patients were reviewed. Out of 664 subjects, 494 cases were excluded from the study by exclusion criteria as presented in Figure 1. Therefore, 170 participants were enrolled in the study. A total of 130 and 40 cases were classified as OS and SS, respectively.

Table 1 showed the baseline characteristics of participants enrolled in the study. The mean age of patients with OS and SS were 53.9±11.7 and 55.0±11.7 years, respectively without statistical significant. Two-thirds (109/170) of both groups reported no underlying disease. Most participants, 75.38% (98/130) with OS were diagnosed of early stage whilst 87.5% (35/40) in SS were diagnosed of advanced stage. One-third (63/170) had clear cell histopathology. Age, BMI, operative time and complication of surgery of both groups were comparable.

High-grade tumor, volume of ascites and EBL in SS were higher than OS with statistical significance. There were 3, 13 and 1 cases whose underwent bladder, bowel and vascular resection, respectively. Both groups had comparable extensive surgery rates. The median level of pre-operative HE4 were 118.60 and 603.45 pmol/L in OS and SS, respectively. OS and SS had average pre-operative CA125 at 146.95 and 814.70 U/L, respectively. Pre-operative serum HE4 and CA125 levels in SS was significantly higher than OS as shown in Table 1.

ROC analysis was generated to evaluate predictive accuracy of HE4 and CA125 expression in discriminating OS and SS as shown in Figure 2. The best cut-off point of HE4 and CA125 less than 170.95 pmol/L and 316.4 U/mL gave predicting OS with area under curve (AUC) at 0.78 and 0.75, respectively. HE4 and CA125 cut-off point had sensitivity, specificity, PPV and NPV at percentage of 60.8/60.8, 87.5/82.5, 94.1/91.9 and 40.7/39.3, respectively for prediction of optimal surgical debulking as presented in Table 2. From binary logistic regression analysis, the accuracy of HE4 and CA125 combination gave sensitivity, specificity, PPV and NPV at percentage of 79.3, 63.6, 96.9 and 17.5, respectively. The AUC for the combination of the two tumor markers was 0.79 (95% CI : 0.72-0.87).

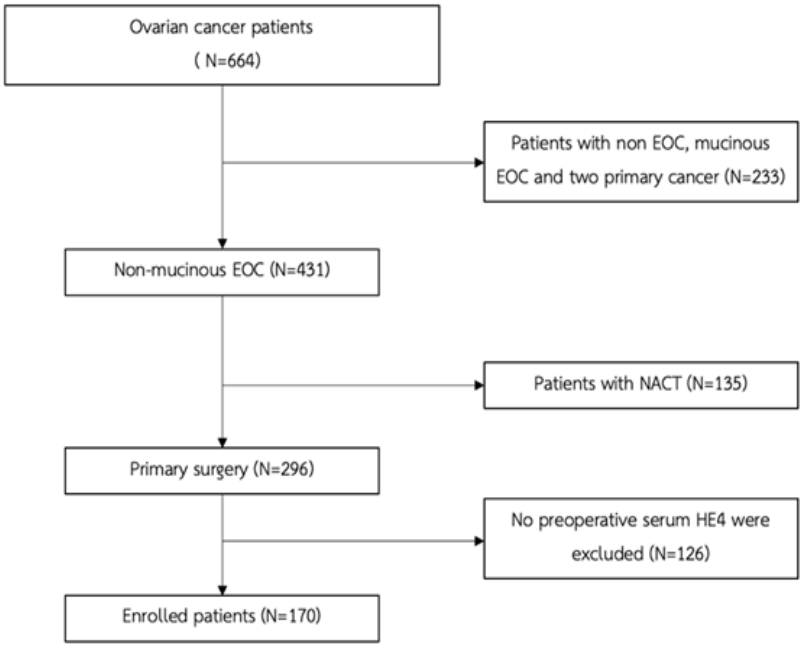

Figure 1. Flow Chart of This Study. EOC, epithelial ovarian cancer; NACT, neoadjuvant chemotherapy; HE4, human epididymis protein 4

Table 1. Baseline Characteristics of the Patients

	Result of primary surgery		p-value
	OS	SS	
Age (year)*	53.9 ± 11.7	55.0 ± 11.7	0.605
BMI (kg/m2) *	23.2 ± 4.2	23.1 ± 4.4	0.913
No underlying disease**	85 (65.4)	24 (60)	0.372
Advanced FIGO stage (III-IV) **	32 (24.6)	35 (87.5)	<0.001
Tumor grade (2/3) **	98 (75.4)	38 (95)	0.006
Clear cell histology**	58 (44.6)	5 (12.5)	0.0002
Operative time (minute)*	181.9 ± 72.9	155.8 ± 83.6	0.088
Ascites (mL)***	0 (0-100)	400 (0-2,400)	<0.001
EBL (mL)***	500 (262.5-1,000)	800 (400-1,750)	0.015
Complication**			0.313
None	98 (75.4)	28 (70)	
GU	5 (3.8)	0 (0)	
GI	6 (4.6)	1 (2.5)	
Hemorrhage	20 (15.4)	11(27.5)	
> 2	1 (0.8)	0 (0)	
HE4 (pmol/L)***	118.6 (60.3-275.4)	603.5 (237.2-1,500)	<0.001
CA125 (U/mL)***	146.9 (38.4-601.1)	814.7 (349.2-1,674.5)	<0.001

* Mean ± standard deviation (SD);** n (%), *** median (interquartile range), BMI, body mass index; OS, optimal primary surgery; SS, suboptimal primary surgery; EBL, estimate blood loss; GU, genitourinary tract; GI, gastrointestinal tract; HE4, human epididymis protein 4; CA125, cancer antigen 125

Table 2. Diagnosis Efficacy of Preoperative Serum HE4 and CA125 Predicting Optimal Primary Surgery

	Cut-off point	Sensitivity*	Specificity*	PPV*	NPV*	AUC (95%CI)
HE4	< 170.95	60.77	87.5	94.05	40.7	0.78 (0.70-0.87)
CA125	< 316.40	60.77	82.5	91.86	39.29	0.75 (0.66-0.83)
HE4&CA125**		79.25	63.64	96.92	17.5	0.79 (0.72-0.87)

*%, **binary logistic regression; PPV, positive predictive value; NPV, negative predictive value; AUC, area under curve; HE4, human epididymis protein 4 (pmol/L); CA125, cancer antigen 125 (U/mL)

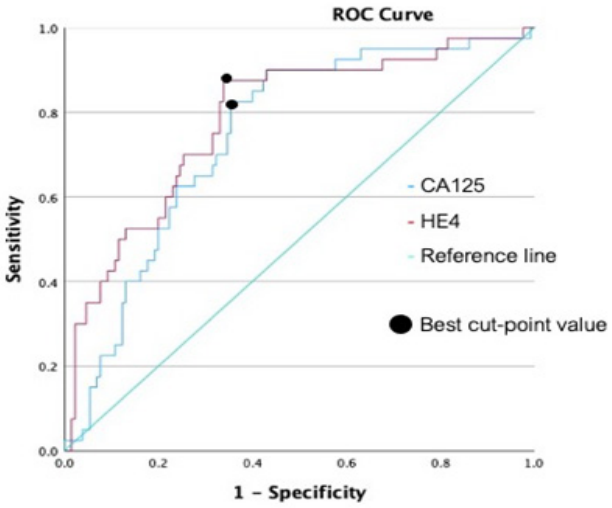

Figure 2. The ROC Curve of Preoperative Serum HE4 and CA125 Predicting Outcome of Primary Surgery. ROC, receiver operating characteristic; HE4, human epididymis protein 4; CA125, cancer antigen 125

Discussion

Among most baseline characteristics were similar between the two groups of enrolled patients in this study. Conversely, FIGO staging, tumor grading, histology, volume of ascites and estimated blood loss (EBL)

Table 3. Comparison of Preoperative HE4 and/or CA125 to Predict Optimal Primary Surgery in Various Studies

		Angioli	Shen	Tang	Paunovic	Feng	Saffarieh	Current
Year		2012	2016	2017	2017	2020	2022	2023
Cases (n)		57	82	90	50	83	101	170
Country		Italy	China	China	Serbia	China	Iran	Thailand
Stage (E/A)		0/57	Oct-72	0/90	0/50	0/83	51/50	130/40
Cut point								
	HE4	262	353.2	473	413	777.1	170	170.9
	CA125	-	1013.4	500	-	313.6	320	316.4
Sensitivity*								
	HE4	86.1	77.4	81	-	48	80	60.8
	CA125	-	80.6	80	-	81	80	60.8
	Combination							79.3
Specificity*								
	HE4	89.5	75	56	-	89	70	87.5
	CA125	-	50	40	-	42	70	82.5
	Combination							63.6
PPV*								
	HE4	93.9	92.3	67	-	71	68	94.1
	CA125	-	86.2	59	-	45	71	91.9
	Combination							96.9
NPV*								
	HE4	77	46.2	73	-	74	81	40.7
	CA125	-	40	65	-	79	82	39.3
	Combination							17.5
AUC								
	HE4	0.86	0.76	0.72	-	0.68	0.82	0.78
	CA125	-	0.63	0.6	-	0.53	0.79	0.75
	Combination							0.79

*%, E/A, early/advanced FIGO stage; PPV, positive predictive value; NPV, negative predictive value; AUC, area under curve; HE4, human epididymis protein 4 (pmol/L); CA125, cancer antigen 125 (U/mL)

showed significant difference between the two groups. Subjects in SS group had more advanced stage, higher tumor grade and a higher proportion of non clear cell histological subtype than OS group. Previous literatures reported the optimal primary surgery among advanced ovarian cancer [5-8] while Shen's and Saffarieh's studies reported both early and advanced ovarian cancer [9, 10]. The subjects in the current study composed of early and advanced ovarian cancer with 130 and 40 cases, respectively. One third (63/170) of the current study and 5 percent (4/82) of Shen's study composed of clear cell histology type of ovarian cancer. The studies of Angioli's, Tang's, Paunovic's, Saffarieh's and Feng's recruited serous histologic type of ovarian cancer at percentage of 47 (27/57), 64 (58/90), 68 (34/50), 53 (54/101) and 81 (68/83), respectively. Advanced ovarian cancer with serous histology had better survival and optimal primary surgery than those with clear cell histology subtype [2].

High pre-operative HE4 and CA125 levels were associated with suboptimal primary surgery. It has been thought that high preoperative serum levels of CA125 are associated with a large tumor burden whilst high serum levels of HE4 are associated with serous or endometrioid histological subtypes of OC [11]. These might be the result of more extensive disease, higher difficulty of complete cytoreduction, greater volume of ascites and EBL among subjects in SS group. In scenario that optimal primary surgery could not be accomplished, NACT should be considered [2]. If we had appropriate prediction tool for optimal primary surgery, there was no need for NACT. This highlights the importance of using predictive factors before initiating treatment in order to foresee surgical outcome.

Cut-off point of HE4 (<170.9 pmol/L) and CA125 (<316.4 U/mL) gave sensitivity, specificity, PPV and NPV at percentage of 60.8/60.8, 87.5/82.5, 94.0/91.9 and 40.7/39.3, respectively. The NPV of each tumor marker was around 40 percent while PPV was between 91 and 94 percent. Combination of both tumor markers gave high PPV (96.9%) and low NPV (17.3%). The high PPV of HE4 and CA125 below cut point gave assurance of nearly hundred percent for surgeon to perform complete primary surgery (removal of all tumor). Combination of HE4 and CA125 had lower NPV than the previous literatures that range from 40 to 80 percent [9, 5, 6, 8, 10]. The PPV from current study had higher than study of Tang, Feng and Saffarieh [6, 8, 10]. While PPV from the current study were comparable to Angioli's and Shen's studies. Percentage of clear cell histology in the current study was higher than Angioli's and Shen's studies [9, 5]. Combination of HE4 and CA125 would be better than HE4 or CA125 alone for prediction of optimal primary surgery. Table 3 compares the diagnostic efficacy of serum HE4 and CA125 in primary optimal surgery of this study to prior studies.

The current study recruited higher percent of clear cell adenocarcinoma than previous literatures. High successful prediction for surgeon to perform optimal primary surgery than choosing NACT. Limitations in this study includes the nature of a retrospective analysis. Furthermore, including patients with early stage (FIGO stage I/II) would likely affect the association between the predictors of interest (HE4 and CA125) and cytoreductive surgery outcome. However, this study showed combination of pre-operative HE4 and CA125 levels tend to predict outcome of primary surgery in patients with non-mucinous EOC especially clear cell adenocarcinoma. The further study should be conducted in the larger sample size of advanced stage and other clinical appearance namely age, BMI, performance status and amount of ascites to develop a valid algorithm in prediction of primary optimal surgery.

In conclusion, our study presented low pre-operative serum HE4 and CA125 levels had significantly related with optimal debulking surgery among non-mucinous EOC especially clear cell adenocarcinoma. The best cut-off point value of HE4 and CA125 for predicting primary optimal surgery were less than 170.95 pmol/L and 316.40 U/mL, respectively with high positive predictive value. Combination of pre-operative serum HE4 and CA125 levels gave 96 percent PPV for predicting complete debulking surgery.

Author Contribution Statement

KP and SO conceptualized and designed the study; KP collected the data, analyzed, interpreted the results and prepared the initial manuscripts. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

Availability of data

All data relevant to this study has been presented in the manuscript. More data requirement was controlled by the institution board according request.

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