RESEARCH ARTICLE

# Modification of Cutoff Values for HE4, CA125, the Risk of Malignancy Index, and the Risk of Malignancy Algorithm for Ovarian Cancer Detection in Jakarta, Indonesia

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## Abstract

**Background: CA125 and HE4 are used in calculating Risk of Malignancy Algorithm (ROMA); and Risk of Malignancy Index (RMI). However, studies showed that normal levels of CA125, and HE4 differ among ethnicities such as between Asians and Caucasians, thus affecting the accuracy of the RMI score and ROMA in predicting ovarian malignancy. This study aimed to determine whether new or modified cutoff values for Ca-125, HE4, the RMI score, and ROMA resulted in a better prediction of malignancy compared with the previous or standard ones. Materials and Methods: Serum level of CA125 and HE4 from 128 patients with diagnosis of ovarian tumor that had been collected before surgery at Cipto Mangunkusumo General Hospital (CMH) in Jakarta from November 2010 until May 2011 were reviewed and analysed. The standard cutoff values of these biomarkers, RMI, and ROMA were modified by using logistic regression model. The modified cutoff values were compared to the standard cutoff values in terms of sensitivity, specificity, and accuracy. Results: The modified cutoff value of CA125, HE4, RMI score and ROMA were 165.2 U/mL, 103.4 pM, 368.7, 28/54. The sensitivity and specificity of the modified cutoff values CA125, HE 4, RMI score and ROMA in differentiating benign from malignant and borderline were 67% and 75,4%; 73.1% and 85.2%; 73.1% and 80.3%; and 77.6% and 86.9%. While the sensitivity and specificity of the standard cutoff value of CA125; HE4; RMI score; and ROMA were 91% and 24.6%; 83.6% and 65%; 80.6% and 65.6%; and 91.0% and 42.6%. The accuracy of modified cutoff values compared with standard cutoff values were: 71.2% *vs* 59.3%, 78.9% *vs* 75% *vs*, 76.5% *vs* 73.4%, and 82% *vs* 67.9%. Conclusions: The new or modified cutoff values of Ca125, HE4, RMI score and ROMA resulted in higher accuracy compared to the previous or standard ones, at the cost of reduced sensitivity.**

**Keywords:** Ovarian cancer - CA125 - human epidydimis protein 4 - ROMA - RMI - tumor marker cutoff values

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## Introduction

Ovarian cancer is the third most common female malignancy in Indonesia, accounts for 4.27 cases in 100.000 women (Aziz, 2009; Wahidin, 2012). As the second most common gynecologic malignancy worldwide, the majority of them are epithelial types (Boyle, 2008; Hennessy, 2009). Since there is no screening method, ovarian cancer is often diagnosed when the patients already have had complaints, or in advanced stages. This condition brings difficulty and complexity of therapy that consequently leads to poorer prognosis (Havrilesky et al., 2008).

There are some biomarkers and scoring systems that are commonly used to predict malignancy of epithelial ovarian tumor (Bian et al., 2013; Yavuzcan et al., 2013) . However, several studies on these biomarkers showed different conclusions. Rosen et al used CA125 as the main biomarker in detecting ovarian malignancy and concluded that additional complementary marker to improve the result it still needed (Rosen, 2005). Other studies showed that the usage of HE4 as well as combination of HE4 and CA125 in ROMA and RMI are more superior than CA125 alone or other markers (Moore et al., 2008; 2009; Lin et al., 2012). Overall, different studies resulted in different diagnostic values (sensitivity, specificity, and accuracy) of each biomarkers (Van Gorp et al., 2011).

Other studies also compared normal values of biomarkers such as CA125 and HE4 from one population to another. CA125 level between Asian and Caucasian healthy women are different (Pauler et al., 2001). There is also a difference in HE4 level between Indian and Malay ethnicity (Mokhtar et al., 2012). The differences in level of these biomarkers among ethnicities could make different results in sensitivity and specificity. Karen et al., suggested an alternative cutoff value for CA125, HE4, and ROMA that results in different sensitivity and specificity values of each markers (Chan, 2013).

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**Table 1. Distribution of Patients Based on Histopathological Types (Total 128)**

| Benign tumor (61) | | Malignant tumor (50) | | Borderline tumor (17) | |
|---|---|---|---|---|---|
| Endometriosis | 26 (42.62%) | Serous cystadenocarcinoma | 19 (38%) | Mucinous | 11 (64.71%) |
| Mucinous cystadenoma | 18 (29.51%) | Endometrioid | 14 (28%) | Serous | 3 (17.65%) |
| Serous cystadenoma | 14 (29.51%) | Mucinous | 8 (16%) | Endometrioid | 3 (17.65%) |
| Seromucinous | 3 (4.92%) | Clear Cell | 7 (14%) | | |
| | | Carcinosarcoma | 2 (4%) | | |

Based on the differences in biomarker levels as described above, this study aims to know whether the new or modified CA125, HE4, RMI, and ROMA cutoff values resulted in higher accuracy compared with the previous or standard ones in predicting malignancy.

## Materials and Methods

*Study population*

We retrospectively collected the data of ovarian tumor patients in Dr. Cipto Mangunkusumo Hospital (CMH) Jakarta from November 2010 to May 2011. We included patients that were diagnosed as ovarian tumor through physical examination and transvaginal ultrasound. Patients with unresectable tumors, non-epithelial histopathological results, history of oophorectomy, ovarian cancer treatment, and pregnancy were excluded from the study.

*Sample collection*

CA125 and HE4 levels from 128 patients that fulfilled the criteria were obtained. The measurement of CA125 and HE4 was done 1 day before surgery. Afterwards, RMI and ROMA scores were calculated based on the CA125 and HE4 levels. Histopathology analysis of the patients was obtained from our pathologist in CMH. The measurements of CA125 and HE4 were conducted using Abbott reagent for Chemiliminescent Microparticle ImmunoAssay (CMIA).

The histopathological results were categorized into benign, borderline, and malignant ovarian tumor, and then compared to the CA125, HE4, RMI and ROMA results.

*RMI and ROMA*

RMI, as described by Tingulstad et al.,is based on the value of CA125 serum, ultrasound morphology (U) and menopause status (M). Ultrasound score = 1 if there is no morphological abnormalities or only one abnormalities, U= 3 if found≥ 2 morphological abnormalities. Menopause status score is M=1 for pre menopause and M=3 for post menopause. Score ≥ 200 were classified as malignant risk.

*RMI=U×M×the value of CA 125*

ROMA used to predict the risk of ovarian malignancy in patients with pelvic masses, so that patients can be stratified as low risk and high risk based on the values of CA125 and HE4. Premenopausal woman is classified as high risk if the probability prediction (PP)> 74%, for postmenopausal women if PP>25.3%.

Prediction index (PI) formula for premenopausal

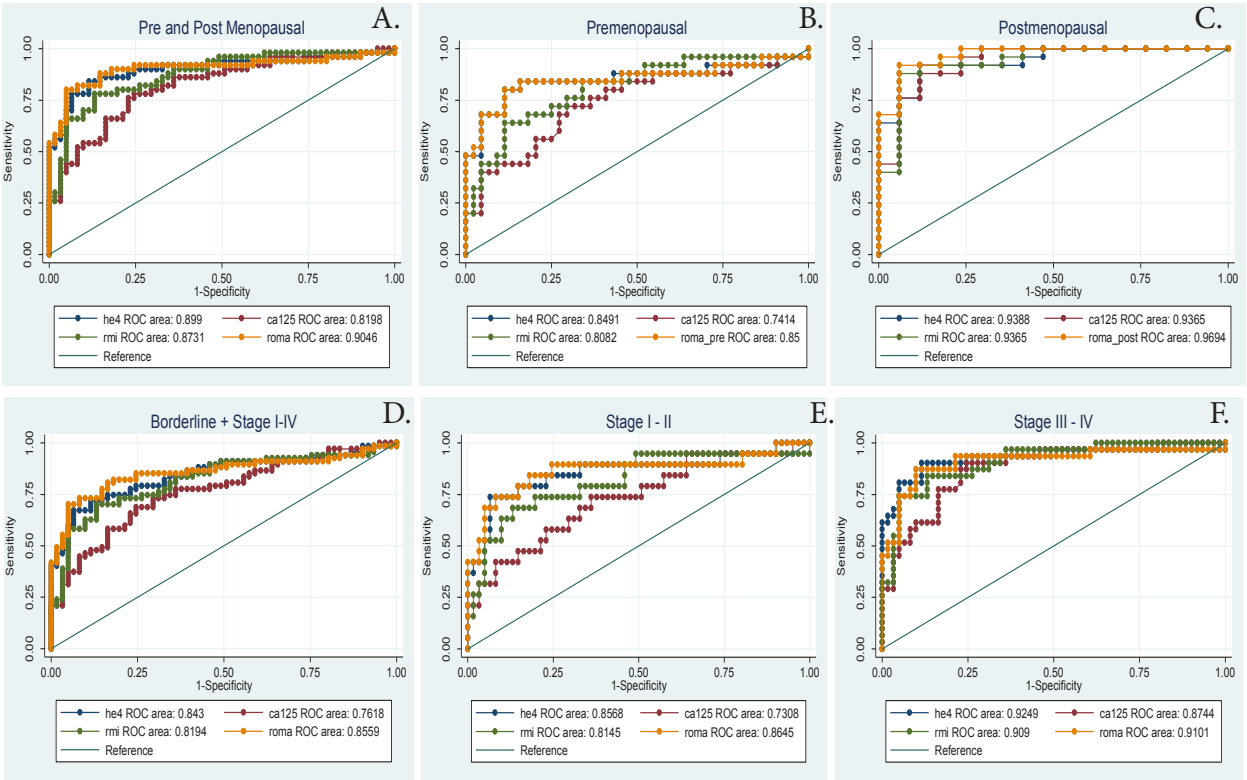


**Figure 1. ROC Curve of CA125, HE4, RMI and ROMA among patients based on menopausal status and FIGO stage. A)** Malignant *vs* Benign on all patients including pre and postmenopause; **B)** Malignant *vs* Benign on premenopausal patients; **C)** Malignant *vs* Benign on postmenopausal patients; **D)** Malignant (including borderline) *vs* Benign on all patients; **E)** Malignant *vs* Benign on stage I-II patients; **F)** Malignant *vs* Benign on stage III-IV patients

**Table 2. Distribution of Age, Menopausal Status, USG Score, CA125, HE4 between Benign and Malignant Ovarian Tumors**

| Variable | Benign n=61 | Malignant n=50 | p value |
|---|---|---|---|
| Age (mean) | 41 | 44 | |
| Menopausal Status | | | |
| Premenopause | 44 (63.77%) | 25 (36.23 %) | p<0.05 |
| Postmenopause | 17 (40.48 %) | 25 (59.52 %) | |
| USG Score | | | |
| 0 | 24 (96.00%) | 1 (4.00%) | p<0.05 |
| 1 | 21 (67.74%) | 11 (32.26%) | |
| 2-5 | 16 (29.09%) | 45 (70.01%) | |
| CA 125 (U/ml) | | | |
| Mean | 195.5 | 1763.47 | p<0.05 |
| Median | 82.5 | 357.45 | |
| Minimum | 8.1 | 13.1 | |
| Maximum | 2441.4 | 9872.3 | |
| HE 4 (pM) | | | |
| Mean | 75.7 | 1338.05 | p< 0.05 |
| Median | 52.3 | 495.45 | |
| Minimum | 29.5 | 26.1 | |
| Maximum | 436.3 | 15000 | |

women: *-12.0+2.38 x LN (Natural Log) (HE4)+0.0626×LN (CA125)*

Prediction index (PI) formula for postmenopausal women: *-8.09+1.04×LN (HE4)+0.732 x LN (CA125)*.

Probability prediction (PP) formula %: *exp (PI)/ [1+exp (PI)]×100*

*Statistical analysis*

Data was analysed using Stata program ver. 9.2, to obtain the value of sensitivity, specificity, PPV, NPV, and accuracy of the markers. ROC curve analysis (DeLong analysis) was used to obtain the value of AUC with 95% confidence interval based on menopausal status and stage of epithelial ovarian cancer.

## Results

From 128 patients, 61 patients (47.66%) had benign ovarian tumour, 50 (39.06%) had malignant tumour, and the other 17 were borderline (13.28%). Those categories are further divided into several types. Included in benign tumor group, there were 26 endometriosis patients (42.62%), 18 (29.51%) mucinous cystadenoma patients, 14 (22.95%) serous cystadenoma patients, and 3(4.92%) patients with seromucinous tumor. In malignant tumor category, respectively there were 19(38%), 14(28%), 8 (16%), 7 (14%), and 2(4%) patients of serous cystadenocarcinoma, endometrioid, mucinous, clear cell carcinoma, and carcinosarcoma. While in borderline group, there were 11(64.71%), 3(17.65%) and 3(17.65%) patients of mucinous, serous, and endometrioid tumors respectively.

The level of CA125 and HE4, ultrasound score, and menopausal status were significantly different between benign and malignant groups. Moreover, median value of HE4 and CA125 serum concentration was significantly higher in patients with EOC than those with benign ovarian tumor (p value <0.05, Table 2).

This study also compared the AUC values among HE4, CA125, RMI and ROMA, that are presented in Figure 1. In the premenopausal group, HE4 and ROMA had the same AUC value of 85.0% (95%CI: 0.73-0.96), while the postmenopausal group had ROMA for the highest AUC value at 96.9% (95%CI: 0.92-1.00), followed by HE4 (93.9%) and simultaneously CA125 and RMI with a same AUC value at 93,6%. The comparison of modified and standard cutoff values were shown in the tables below. In the upper part of Table 3, borderline cases were excluded from the analysis, while in lower part of Table 3, borderline cases were included into the malignant group.

Benign *vs* Malignant analysis of HE4, CA125, RMI and ROMA using modified cutoff values shows a higher specificity and accuracy values than those with standard cutoff values at 85.2%, 75.4%, 80.3%, 86.9% for specificity, and 85.6%, 76.5%, 80.2%, 87.4% for accuracy (see Table 3). From analysis that included borderline cases into malignant group, it can also be seen that modified cutoff values results in higher specificity and accuracy. On the other hand, the use of standard cutoff values results in

**Table 3. Diagnostic Values of HE4, CA125, RMI and ROMA using Standard and Modified Cutoff Values**

| Marker Cutoff Types | HE4 | | CA125 | | RMI | | ROMA | |
|---|---|---|---|---|---|---|---|---|
| | Standard | Modified | Standard | Modified | Standard | Modified | Standard | Modified |
| Benign vs Malignant | | | | | | | | |
| Cutoff Value | 70 | 103.4 | 35 | 165.2 | 200 | 368.7 | 7.4/25.3 | 28/54.8 |
| Sensitivity | 90.0% | 86.0% | 96.0% | 78.0% | 88.0% | 80.0% | 94.0% | 88.0% |
| Specificity | 65.6% | 85.2% | 24.6% | 75.4% | 65.6% | 80.3% | 42.6% | 86.9% |
| PPV | 68.2% | 82.7% | 51.1% | 72.2% | 67.7% | 76.9% | 57.3% | 84.6% |
| NPV | 88.9% | 88.1% | 88.2% | 80.7% | 87.0% | 83.1% | 89.7% | 89.8% |
| LR+ | 2.61 | 5.83 | 1.27 | 3.17 | 2.56 | 4.07 | 1.64 | 6.71 |
| LR- | 0.15 | 0.16 | 0.16 | 0.29 | 0.18 | 0.25 | 0.14 | 0.14 |
| Accuracy | 76.5% | 85.6% | 56.7% | 76.5% | 75.6% | 80.2% | 65.7% | 87.4% |
| Benign vs Malignant+Borderline | | | | | | | | |
| Cutoff Value | 70 | 103.4 | 35 | 165.2 | 200 | 368.7 | 7.4/25.3 | 28/54.8 |
| Sensitivity | 83.6% | 73.1% | 91.0% | 67.2% | 80.6% | 73.1% | 91.0% | 77.6% |
| Specificity | 65.6% | 85.2% | 24.6% | 75.4% | 65.6% | 80.3% | 42.6% | 86.9% |
| PPV | 72.7% | 84.5% | 57.0% | 75.0% | 72.0% | 80.3% | 63.5% | 86.7% |
| NPV | 78.4% | 74.3% | 71.4% | 67.6% | 75.5% | 73.1% | 81.3% | 77.9% |
| LR+ | 2.43 | 4.96 | 1.21 | 2.73 | 2.34 | 3.72 | 1.59 | 5.92 |
| LR- | 0.25 | 0.31 | 0.36 | 0.43 | 0.29 | 0.33 | 0.21 | 0.26 |
| Accuracy | 75.0% | 78.9% | 59.3% | 71.2% | 73.4% | 76.5% | 67.9% | 82.0% |

a higher sensitivity than the modified one.

## Discussion

In this study, the sensitivity of CA125 to detect EOC based on standard cutoff (35 U/ml) was very high at 96%, with a very low specificity value at 24.6%; and so does HE4 with 90% sensitivity and 65.6% specificity. A low specificity means that there is a possibility of a lot number of patients got over treatment.The very low value of CA125 specificity in this study was because the mean and median values of CA125 from all benign tumour samples in this research were above the value of standard cutoff. However, this finding was different from a study by Van Gorp et al (2011) where the sensitivity for both CA125 and HE4 were lower than the specificity.

From the statement above, it could be concluded that by using the same biomarkers and cutoff values, different study might results in different sensitivity and specificity results (Chan, 2013). It is because different studies might have different characteristic in their population. People with different ethnicity tend to have different level of biomarkers. Caucasians women tend to have higher CA125 level compared to Asian and African women (Pauler, 2001). Another study also showed that Indian and Malay healthy women had a different HE4 level (Mokhtar, 2012).

Havrilesky, et al. and Gorp et al. showed that a same cutoff values might results in different diagnostic values on a study population with different stages of disease (Havrilesky, 2008; Van Gorp, 2011). This finding could also be seen in this study, in figure 1, where the AUC of every biomarkers and scorings are different in early and advance stage of EOC. Differences in diagnostic values could also be found among women with different menopausal status (Figure 1).

Therefore, our study tried to determine a new cutoff value to improve the sensitivity and specificity values of the biomarkers thus improving their accuracy as well. This study sought the modified cutoff values of CA125 and HE4 tumour markers that was obtained from the calculation of logistic regression analysis of levels of CA125 and HE4 of the entire sample. Afterwards, using logistic regression analysis, a graph of sensitivity and specificity was made to obtain the cutoff value for optimal sensitivity and specificity. The goal was to obtain the maximal accuracy value (minimal negative false and positive false).

The modified cutoff value found in this research was 165.2 u/mL for CA125 and 103.4 pM for HE4 . The standard cutoff value of HE4 at 70 pmol/L was determined based on a study by Moore et al (2008) and a recommendation from insert KIT ARCHITECT HE4 reagent that was used in this research.

Chang et al., (2011) reported that along with the increase of the cutoff value of either CA125 or HE4, a higher specificity will be obtained; where their cutoff value of CA125 and HE4 on 95% specificity was 127.2 u/mL and 102.6 pmol/L, respectively. Furthermore, at 98% specificity, the obtained cutoff value was 325.5 u/mL for CA125 and 150.2 pmol/L for HE4.

Studies conducted by Moore at al (2008), Huhtinen at al (2009), Nolen et al (2010), Holcomb et al (2011), and Chang et al (2011), combined the use of CA125 and HE4 stated that a combination of CA125 and HE4 to further improve the diagnostic capability to differentiate malignant and benign tumors among patients with adnexal masses before surgery.

Moore et al. (2009) introduced a new algorithm known as ROMA (Risk of Ovarian Malignancy Algorithm) to predict ovarian malignancy, by combining the results of CA125 and HE4 using mathematical calculations (Moore, 2008; 2009; Huhtinen, 2009; Nolen, 2010; Chang, 2011; Holcomb, 2011). Our study set a new modified cutoff values for RMI at 368.7 u/mL and ROMA at 28%/54.8%.

When our study excluded the borderline group in the analysis, we found that HE4 and RMI as EOC predictors have a higher accuracy value than ROMA and CA125 on the standard cutoff, while HE4 and ROMA have a better accuracy value than RMI and CA125 on the modified cutoff values (Table 3). However, Table 3 also showed that an modified cutoff values improves the specificity and accuracy of every biomarkers, although it decreases the sensitivity value.

This study showed the benefit of modified cutoff value compared to the standard ones from several biomarkers/methods including CA125, HE4, RMI and ROMA scoring in our patients. These modified cutoff values might result in different outcomes in different populations, since this study was conducted in Indonesia.

In conclusion, modified cutoff values resulted higher accuracy in predicting risk of malignancy compared with the standard cutoff values. They are alsoimportant in determining the diagnostic accuracy of a marker in our ethnic group Indonesian people. The previous or standard cutoff value from manufacturer might result in different sensitivity, specificity and accuracy from one study to another (Chan, 2013). It can be influenced by the ethnics, staging, or manopausal status (Pauler, 2001; Mokhtar, 2012).

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