**TITLE PAGE**

**Title:** Comparison of Risk of Malignancy Index (RMI), CA125, HE4, Risk of Ovarian Malignancy Algorithm (ROMA) and Ultrasound Score for Predictive Ovarian Malignancy in Women with a Pelvic Mass

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**COMPARISON OF RISK OF MALIGNANCY INDEX (RMI), CA125, HE4, RISK OF OVARIAN MALIGNANCY ALGORITHM (ROMA) AND ULTRASOUND SCORE FOR PREDICTIVE OVARIAN MALIGNANCY IN WOMEN WITH A PELVIC MASS**

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**ABSTRACT**

**Background:** Ovarian cancer is the third most common of gynecologic cancer and the second leading cause of gynecologic cancer-related death. Preoperative prediction of ovarian cancer plays a critical role in management of women presenting with a pelvic or ovarian mass and has impact on survival outcome.

**Objective**: To comparison the performance of risk of malignancy index (RMI), cancer antigen-125 (CA125), human epididymis protein 4 (HE4), risk of ovarian malignancy algorithm (ROMA) and Sassone ultrasound score in prediction of ovarian cancer in patients presenting with a pelvic or ovarian mass.

**Materials and methods:** Thiswas a prospective cohort study in women over 18 years old clinical diagnosed pelvic or ovarian masses undergoing elective surgery at Rajavithi Hospital between January, 2012 and December, 2012. Pelvic ultrasonography, serum CA125 and HE4 levels were executed preoperatively, then the Sassone score, RMI and ROMA values were calculated. The performance of all five parameters for discrimination was analyzed from area under the Receiver Operating Characteristic (ROC) curve.

**Results**: A total of 260 women were evaluated, 186 in non-cancer group (158 benign diseases and 28 borderline tumors) and 74 in cancer group (66 epithelial ovarian cancers (EOCs) and 8 non-EOCs). The resultant accuracy values using the area under ROC curves for RMI, CA125, HE4, ROMA and Sassone ultrasound score to distinguish between ovarian cancer versus non-cancer diseases showed 87.61%, 80.59%, 82.36%, 86.16% and 77.11%, respectively. The RMI, ROMA and HE4 level had the highest accuracy in premenopausal women (AUC 85.57%, 84.37% and 84.36%, respectively); whereas the RMI level had the highest accuracy in postmenopausal women (AUC 87.85%) but the HE4 level appeared in the lowest accuracy (AUC 77.08%). Patients with clear cell carcinoma and non-EOCs revealed a high number of false negative cases in all of five parameters.

**Conclusions**: RMI was the most accurate method but ROMA showed no significant difference in performance with RMI in discrimination between non-cancer diseases and ovarian cancer. HE4 level might have a limitation in postmenopausal women. In addition, clear cell carcinoma and non-EOC patients were the defect of all five parameters.

**Key words:** pelvic mass, ovarian cancer, Risk of Malignancy Index, CA 125, HE 4, Risk of Ovarian Malignancy Algorithm, Sassone ultrasound score

**INTRODUCTION**

Ovarian cancer is the third most common of gynecologic cancer and the second leading cause of gynecologic cancer-related death. In 2008, 224,747 new cases were diagnosed and 140,163 women died of the disease worldwide ([1](#_ENREF_1)). The National Institutes of Health (NIH) Consensus Development Conference Statement estimates that 13-21% of patients with a pelvic mass will be diagnosed with an ovarian cancer ([2](#_ENREF_2)). Simultaneously, approximately 70% of ovarian cancer is diagnosed in an advanced stage. The utmost important for long-term survival in women with ovarian cancer is cytoreductive surgery with optimal debulking ([3-5](#_ENREF_3)). In addition, if these patients is operated on by surgeons specializing in the management of ovarian cancer or gynecologic oncologists, the surgical outcome is superior and the overall survival is increased ([6](#_ENREF_6)). Therefore, pre-operative differentiation of ovarian cancer plays a critical role in management of women presenting with a pelvic or ovarian mass and has affect on survival.

Several diagnostic imaging studies for pelvic or ovarian mass have been reported such as ultrasonography (US), computed tomography scan or magnetic resonance imaging. Gray scale US plays a major basic role in general gynecologic practice and frequently is used to assess the risk of malignancy. An extensive number of US studies of ovarian tumors promote establishing pattern recognition of the US features to predict malignancy. Classically, the multiparametric model of US score developed by Sassone et al ([7](#_ENREF_7)) was the most evaluated prediction model.

Even though serum biomarker of cancer antigen-125 (CA125) in commonly applies to predict the presence of a malignancy in women with a pelvic mass, the CA125 measurement has some restrictions. In patients with early-stage ovarian cancer, the serum CA125 level is elevated in less than half of those ([8](#_ENREF_8)). Approximately 20% of ovarian cancer patients, meanwhile, have normal or only marginally elevated serum CA125 ([8](#_ENREF_8)). Furthermore, many benign gynecologic disorders and medical conditions have an elevated serum CA125 level resulting in the decreased performance ([9](#_ENREF_9)).

The combination of serum CA125 level and US parameters improves the accuracy and validity for prediction of the presence of ovarian cancer in patients with a pelvic mass. In 1990, the Risk of Malignancy Index (RMI) was emerged by Jacobs et al ([10](#_ENREF_10)) which was referred to as RMI I. The RMI is presently the accurate tool for stratifying patients into high and low risk groups. Currently, the Royal Thai College of Obstetricians and Gynecologists (RTCOG) recommends using RMI I for preoperative evaluation to differentiation between a benign and malignant pelvic mass for optimal referral and patient care.

Several biomarkers have been discovered to find the additive markers that can distinguish between benign and malignant ovarian tumors. In recent years, Human Epididymis protein 4 (HE4) seems to be a promising biomarker for ovarian cancer ([11](#_ENREF_11), [12](#_ENREF_12)). HE4 is N-glycosylated protein with low expression in normal tissue, higher in non-ovarian cancer and with the highest expression in ovarian cancer ([13](#_ENREF_13)). HE4 has been reported well performance as CA125 for detection of ovarian malignancy. In 2008, Moore et al ([12](#_ENREF_12)) developed the risk of ovarian malignancy algorithm (ROMA), a dual biomarker-based algorithm combining serum CA125 and HE4. ROMA has been shown to better predict the presence of an ovarian malignancy with the high sensitivity and specificity in several studies ([14-17](#_ENREF_14)). However, no study of the comparison of the performance of ROMA and serum HE4 compared with serum CA125, RMI and US score in Asian women had been reported.

The purpose of the present study was to comparison the performance of risk of malignancy index (RMI), cancer antigen-125 (CA125), human epididymis protein 4 (HE4), risk of ovarian malignancy algorithm (ROMA) and Sassone ultrasound score in prediction of ovarian cancer in patients presenting with a pelvic or ovarian mass.

**MATERIALS AND METHODS**

This was a prospective cohort study conducted at the Department of Obstetrics and Gynecology of Rajavithi Hospital during January 1, 2012 and December 31, 2012. After obtaining Institutional Review Board approval, all women over 18 years of age clinically diagnosed pelvic or ovarian mass undergoing elective gynecologic surgery were enrolled and all patients were asked to give written informed consent.

All patients had radiologic imaging by pelvic US within 6 weeks prior to surgery to document the presence of a pelvic or ovarian mass. As routing practice in Rajavithi Hospital, all patients who had scheduled to elective surgery were admitted for the preoperative preparation at least 24 hours prior to operation, then clinical data were collected and the blood tests of CA125 and HE4 were performed preoperatively. The exclusion criteria included women with pregnancy, previous history of ovarian cancer or any known malignancies, and previous history of adnexal surgery.

Postmenopausal women were defined in those more than 45 years of age with cessation of menstrual bleeding more than one year or those over 55 years of age. If the menopausal status could not be identified from the clinical data, the FSH level was tested using to assign the menopausal status at the cutoff value of 25 IU/L.

Patients received pelvic US executing by single operator (Siricharoenthai S) blinded the results of serum biomarker levels and using the Voluson E8 (GE Medical System Kretztechnik GmbH & Co OHG, Austria). The examinations of the following morphologic parameters; inner wall structure, wall thickness, wall septa, and echogenicity were carried out via transabdominal or transvaginal mode as appropriate. A point score was given for each parameter according to the criteria of Sassone et al ([7](#_ENREF_7)) as shown in table 1 and summing the score of individual parameter was calculated for the total score. The cutoff value for distinction between benign diseases and ovarian cancer was 9.

**Table 1** Sassone scoring system

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Score | Wall structure | Wall thickness | Septum | Echogenicity |
| 1 | Smooth | < 3 mm | Absent | Anechoic |
| 2 | Irregular < 3 mm | > 3 mm | < 3 mm | Hypoechoic |
| 3 | Papilla > 3 mm | Solid | > 3 mm | Hypoechoic with hyperechoic spots |
| 4 | Solid |  |  | Mixed |
| 5 |  |  |  | Hyperechoic |

Five ml of blood sample was obtained by peripheral venous puncture within 48 hours prior to surgery and processed immediately or stored at -20oC until needed. Clotted blood tubes were centrifuged at 800 g (gravitational force) for 10 minutes then serum was separated. Serum biomarker concentrations were analyzed using Elecsys HE4 and Elecsys CA125 II (Cobas 6000 analyzer series, Roche Diagnostics, Indianapolis, IN) according to the manufacturer’s instructions. For CA125, the cutoff values were 200 U/mL for premenopausal and 35 U/mL for postmenopausal women, as recommended by the American College of Obstetricians and Gynecologists (ACOG) ([18](#_ENREF_18)). The cutoff values for HE4 were 70 pM/L for premenopausal and 140 pM/L for postmenopausal women, per the manufacturer’s instructions.

RMI was calculated as the product of the serum CA125 level, the ultrasound parameters and the menopause status according to the criteria described by Jacobs et al. ([10](#_ENREF_10)) as follows: RMI = U x M x CA125, where U = the ultrasound score, M = menopausal status, and CA125 = the level of this marker. U was calculated as follows: multilocularity, solid areas, bilaterality, ascites and intraabdominal metastases each scored one point and total scores of 0, 1 and > 2 points yielded U values of 0, 1 and 3, respectively. Menopausal status was coded 1 for premenopausal and 3 from postmenopausal. The cutoff RMI value for differentiating between benign versus malignant masses was 200, as proposed by Jacobs et al ([10](#_ENREF_10)).

ROMA were constructed by using serum CA 125 and HE4 levels accompanied with menopausal status. A predictive index (PI) was calculated by the following equations described by Moore et al ([12](#_ENREF_12)), premenopausal women: PI = -12 + [2.38 × Ln(HE4)] + [0.0626 × Ln(CA 125)] and postmenopausal women: PI = -8.09 + [1.04 × Ln(HE4)] + [0.732 × Ln(CA 125)]. Then the ROMA value (predictive value) was calculated by the following equation, ROMA (%) = [ePI/(1+ePI)] x 100.The cutoff values for ROMA were 11.4% for premenopausal and 29.9% for postmenopausal women, per the manufacturer’s instructions.

All patients underwent to operation and comprehensive surgical staging including abdominal peritoneal cytology, total abdominal hysterectomy, bilateral salpingooophorectomy, omentectomy and pelvic and para-aortic lymph node dissections was performed in case of suspected ovarian cancer. Fertility-sparing surgery was the alternative option in apparently stage I ovarian cancer patients who desired to become pregnant conceive or had young age. If full comprehensive staging and cytoreduction had not possible, the patients were offered biopsy for diagnosis and 3 cycle of neoadjuvant chemotherapy followed by cytoreductiive surgery was performed. Patient participation in the study was concluded once the final surgical pathology reports were obtained. All surgical specimens were reviewed by Yanaranop M. and Nakrangsee S.

Sample size calculation was based on the formula for comparing areas under the Receiver Operating Characteristics (ROC) curves proposed by Hanley et al ([19](#_ENREF_19)) using PASS 2005 (LLC, Kaysville, Utah). The area under ROC curves from the study of Moore et al ([18](#_ENREF_18)) was used for calculation and at least 254 subjects were computed. From expected 10% dropout, 282 subjected were finally required.

Statistical analysis was undertaken using STATA 10 (StataCorp, College Station,TX). The baseline characteristics of participants, described using frequency and percentage for categorical data and mean, standard deviation, median and range for continuous data, were compared using the Student t-test, Mann-Whitney U test and Chi-square test. To investigate the parameters (biomarkers, ultrasonographic findings and predictive scores) for prediction of ovarian malignancy, the crude odds ratio and theirs 95% confidence interval were expressed. Using the pathologic diagnosis as the clinical classification reference, ROC curve was plotted and area under the curve (AUC) was calculated to compare the accuracy of each parameter for predicting malignant ovarian tumors, as well as the sensitivity, specificity, positive predictive value and negative value were carried out. The optimal cutoff value was calculated using Youden Index method ([20](#_ENREF_20)). A probability value of less than 0.05 was considered statistically significant.

**RESULTS**

During the study period, 280 women with clinically diagnosed pelvic or ovarian mass having a planed surgical intervention were prospectively enrolled. Two patients had pregnancy, 5 patients with history of previous adnexal surgery, one patient with history of breast cancer and 12 patients without HE4 biomarker results were excluded from the study. Finally, a total of 260 participants were included as summarized in Figure 1 and divided into non-cancer group (158 benign diseases and 28 borderline tumors) and cancer group (66 epithelial ovarian cancers or EOCs and 8 non-EOCs).

**Figure 1** Flow chart to illustrate patient enrollment and subsequent exclusion of the patient.

Total women > 18 yr, presenting a pelvic or ovarian mass undergoing elective gynecologic surgery in Rajavithi Hospital during January 1, 2012 and December 31, 2012

(N = 280)

Study participants

(N = 260)

Excluded 20 patients

* 2 patients: pregnancy
* 5 patients: history of previous adnexal surgery
* 1 patients: history of previous breast cancer
* 12 patients: no serum HE4 results

Non-cancer group

(N = 186)

Cancer group

(N = 74)

The baseline characteristics of participants were summarized in Table 2. The mean age was 46.33 and 52.83 years for those with non-cancer and ovarian cancer respectively revealing a statistically significant difference (p < 0.001). There were no statistically significant differences in term of body weight, height, body mass index (BMI), parity and underlying disease. However, the postmenopausal status presented significantly in those with ovarian cancer compared to non-cancer (62.16% vs. 35.38%, p < 0.001).

**Table 2** Baseline characteristics ofpatients presenting with a pelvic or ovarian mass

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Variables | | Total  (n=260) | | Non-cancer  (n=186) | | Cancer  (n=74) | | p value |
| Age (years)a, mean (SD) | | 48.18 | (14.19) | 46.33 | (14.83) | 52.82 | (12.27) | <0.001\* |
| Weight (kg)a, mean (SD) | | 58.48 | (12.17) | 58.85 | (12.63) | 57.43 | (10.92) | 0.397 |
| Height (cm)a, mean (SD) | | 154.62 | (6.11) | 154.80 | (6.16) | 154.16 | (5.98) | 0.450 |
| BMI (kg/m2)a, mean (SD) | | 24.44 | (4.78) | 24.57 | (5.10) | 24.11 | (3.90) | 0.487 |
| Parityb, median (range) | | 1 | (0-10) | 1 | (0-8) | 1 | (0-10) | 0.561 |
| Underlying diseasesc (%) | | 90 | (34.62) | 62 | (33.33) | 28 | (37.84) | 0.491 |
|  | Hypertension | 62 | | 44 | | 18 | |  |
|  | Diabetic mellitus | 22 | | 16 | | 6 | |  |
|  | Other | 20 | | 14 | | 6 | |  |
| Menopausec (%) | |  |  |  |  |  |  | <0.001\* |
|  | Premenopause | 148 | (56.92) | 120 | (64.52) | 28 | (37.84) |  |
|  | Postmenopause | 112 | (43.08) | 66 | (35.48) | 46 | (62.16) |  |

Abbreviation: SD = Standard deviation, BMI = Body mass index

aIndependent t-test, bMann-Whitney U test, cChi-square test, \*Significant at p < 0.05

Distribution of histopathologic diagnosis was described in Table 3. There were 8 patients diagnosed with non-EOCs (2 granulosa cell tumors, 1 yolk sac tumor, 1 fibrosarcoma, 1 metastatic colorectal adenocarcinoma, 1 metastatic neuroendocrine tumor and 2 malignant lymphomas). In EOC patients, there were 30 stage I, 13 stage II, 21 stage III, and 2 stage IV.

**Table 3** Distribution of pathologic diagnosis for all cases

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Pathology | | | Premenopause (%) | | Postmenopause (%) | | Total (%) | |
| Benign diseases | | |  |  |  |  |  |  |
|  | Endometriotic cysts | | 43 | (29.05) | 5 | (4.46) | 48 | (18.46) |
|  | Teratomas | | 21 | (14.91) | 9 | (8.04) | 30 | (11.54) |
|  | Serous cystadenomas | | 10 | (6.76) | 11 | (9.82) | 21 | (8.08) |
|  | Mucinous cystadenomas | | 15 | (10.14) | 16 | (14.29) | 31 | (11.92) |
|  | Functional/Simple/Paratubal cysts | | 2 | (1.35) | 5 | (4.46) | 7 | (2.69) |
|  | Tuboovarian abscess | | 5 | (3.38) | 2 | (1.79) | 7 | (2.69) |
|  | Leiomyomas | | 6 | (4.05) | 1 | (0.89) | 7 | (2.69) |
|  | Fibrothecomas | | 1 | (0.68) | 2 | (1.79) | 3 | (1.15) |
|  | Brenner tumors | | 0 | (0.00) | 2 | (1.79) | 2 | (0.77) |
|  | Pseudocysts | | 1 | (0.68) | 1 | (0.89) | 2 | (0.77) |
| Borderline tumors | | |  |  |  |  |  |  |
|  | Serous borderline tumors | | 2 | (1.35) | 2 | (1.79) | 4 | (1.54) |
|  | Mucinous borderline tumors | | 14 | (9.46) | 9 | (8.04) | 23 | (8.85) |
|  | Endometrioid borderline tumors | | 0 | (0.00) | 1 | (0.89) | 1 | (0.38) |
| Cancer | | |  |  |  |  |  |  |
|  | EOC | | 25 | (16.89) | 41 | (36.60) | 66 | (25.38) |
|  |  | Serous carcinoma | 6 | (4.05) | 7 | (6.25) | 13 | (5.00) |
|  |  | Mucinous carcinoma | 1 | (0.68) | 7 | (6.25) | 8 | (3.08) |
|  |  | Endometrioid carcinoma | 5 | (3.38) | 9 | (8.04) | 14 | (5.38) |
|  |  | Clear cell carcinoma | 8 | (5.41) | 11 | (9.82) | 19 | (7.31) |
|  |  | Mixed epithelial carcinoma | 4 | (2.70) | 4 | (3.57) | 8 | (3.08) |
|  |  | Adenocarcinoma, NOS | 1 | (0.68) | 3 | (2.68) | 4 | (1.54) |
|  | Non-EOC | | 3 | (2.03) | 5 | (4.46) | 8 | (3.08) |
| Overall | | | 148 | (56.92) | 112 | (43.08) | 260 | (100) |
|  | Benign | | 104 | (70.27) | 54 | (48.21) | 158 | (60.77) |
|  | Borderline tumor | | 16 | (10.81) | 12 | (10.71) | 28 | (10.77) |
|  | Cancer | | 28 | (18.92) | 46 | (41.07) | 74 | (28.46) |

Abbreviation: EOC = Epithelial ovarian carcinoma, NOS = not otherwise specific

Analysis of the predictive effect for ovarian malignancy of biomarker levels, ultrasonographic findings and three predictive scores in women presenting with a pelvic or ovarian mass was showed in table 4. The serum CA125 and HE4 levels accompanied with ROMA, RMI and Sassone score were significantly associated with predictive ovarian malignancy (p < 0.001). In addition, the significant ultrasonographic findings associated with diagnosis of ovarian cancer were solid area, ascites and intraabdominal metastasis (p < 0.001).

**Table 4** Bivariate analysis of biomarker levels, ultrasonographic findings and predictive scores for predicting ovarian malignancy

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Variables | | Total  N | Ovarian cancer | | | | |
| % |  | OR | 95%CI | p value |
| CA-125, U/mL | | 260 | 28.46 |  | 1.004 | 1.002-1.005 | <0.001\* |
| HE4, pM/L | | 260 | 28.46 |  | 1.003 | 1.002-1.005 | <0.001\* |
| ROMA, % | | 260 | 28.46 |  | 1.05 | 1.04-1.06 | <0.001\* |
| RMI | | 260 | 28.46 |  | 1.001 | 1.000-1.002 | <0.001\* |
| Sassone score | | 260 | 28.46 |  | 1.52 | 1.34-1.72 | <0.001\* |
| US findings | |  |  |  |  |  |  |
|  | Multiloculation | 138 | 33.33 |  | 1.68 | 0.97-2.91 | 0.629 |
|  | Solid area | 105 | 55.24 |  | 10.72 | 5.63-20.43 | <0.001\* |
|  | Bilaterality | 47 | 21.28 |  | 0.63 | 0.29-1.34 | 0.217 |
|  | Ascites | 45 | 60.00 |  | 5.36 | 2.72-10.57 | <0.001\* |
|  | Intraabdominal metastasis | 25 | 64.00 |  | 5.43 | 2.28-12.93 | <0.001\* |
| Sum of US findings | | 260 | 28.46 |  | 2.75 | 2.01-3.77 | <0.001\* |

| | | | | | | | | |

0.5 1 2 3 4 5 6 7 8 9 10

Abbreviation: OR = Odds ratio, CI = Confident interval, CA-125 = Carcinoma antigen 125, HE4 = Human epidermis protein 4, ROMA = Risk of Ovarian Malignancy Algorithm, US = Ultrasonography, RMI = Risk of Malignancy Index

\*Significant at p < 0.05

Evaluation of the performance of distinction of non-cancer and ovarian cancer presented in table 5 and figure 2, the ROC curves for RMI, CA125, HE4, ROMA and Sassone score were constructed to compare the accuracy of these parameters. The greatest AUC was associated with RMI value (87.61%), as compared to the AUC for ROMA value (86.16%), HE4 level (82.36%), CA125 level (80.59%) and Sassone score (77.11%). Slight difference was observed in AUC for ROMA value compared to RMI value. In contrary, AUC for both CA125 level and Sassone score showed significantly lower than RMI value (p = 0.02 and 0.03, respectively). Meanwhile, the comparison of non-cancer and EOC patients found that the performance of ROMA and RMI value showed a best AUC of ROC (88.45% and 88.40%, respectively). At the optimal cutoff value, the RMI values had the highest sensitivity of 83.78% and negative predictive value (NPV) of 92.4% for distinguishing non-cancer from ovarian cancer.

In patients with stage I EOC compared with non-cancer, RMI showed similar high AUC as ROMA (82.89% and 81.79%) and significantly better than serum CA125 level (70.89%). While differentiation between patient with stage II-IV EOC and non-cancer conditions, RMI, ROMA and HE4 levels revealed similar high AUC (93.00%, 94.00% and 93.14%, respectively)

**Table 5** Performance of Risk of Malignancy index (RMI), CA125, HE4, Risk of Ovarian Malignancy Algorithm (ROMA) and Sassone score for prediction of ovarian cancer

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patients (N) | Tests | ROC | | | Optimal cutoff value | Se (%) | Sp (%) | PPV (%) | NPV (%) |
| AUC | (95%CI) | p |
| Non-cancer (186) vs. Cancer (74) | RMI | 87.61 | (82.71-92.52) |  | 169.11 | 83.78 | 78.49 | 60.78 | 92.41 |
| CA125 | 80.59 | (74.17-87.02) | 0.002\* | 109.50 | 71.62 | 80.11 | 58.89 | 87.65 |
| HE4 | 82.36 | (76.17-88.55) | 0.100 | 69.70 | 83.43 | 70.97 | 53.04 | 91.03 |
| ROMA | 86.16 | (80.87-91.44) | 0.553 | 25.61 | 79.73 | 80.65 | 62.11 | 90.91 |
| Sassone | 77.11 | (70.96-83.27) | 0.003\* | 10.00 | 63.51 | 82.80 | 59.49 | 85.08 |
| Non-cancer (186) vs. EOC (66) | RMI | 88.40 | (83.35-93.45) |  | 169.11 | 84.85 | 78.49 | 58.33 | 93.59 |
| CA125 | 82.09 | (75.63-88.55) | 0.007\* | 109.50 | 74.24 | 80.11 | 56.98 | 89.76 |
| HE4 | 84.30 | (78.29-90.31) | 0.203 | 69.70 | 83.33 | 70.97 | 50.46 | 92.31 |
| ROMA | 88.45 | (83.76-93.14) | 0.983 | 26.04 | 80.30 | 81.18 | 60.23 | 92.07 |
| Sassone | 75.84 | (69.27-82.41) | <0.001\* | 10.00 | 60.61 | 82.80 | 55.56 | 85.56 |
| Non-cancer (186) vs. Stage I EOC (30) | RMI | 82.89 | (74.65-91.12) |  | 169.11 | 76.67 | 78.49 | 36.51 | 95.42 |
| CA125 | 70.89 | (59.84-81.93) | <0.001\* | 123.30 | 56.67 | 82.26 | 34.00 | 92.17 |
| HE4 | 73.70 | (63.29-84.11) | 0.098 | 64.12 | 80.00 | 62.90 | 25.81 | 95.12 |
| ROMA | 81.79 | (74.08-89.50) | 0.735 | 14.17 | 90.00 | 63.44 | 28.42 | 97.52 |
| Sassone | 72.15 | (63.32-80.98) | 0.052 | 10.00 | 53.33 | 82.80 | 33.33 | 91.67 |
| Non-cancer (186) vs. Stage II-IV EOC (36) | RMI | 93.00 | (87.70-98.29) |  | 342.00 | 86.11 | 87.63 | 57.41 | 97.02 |
| CA125 | 91.42 | (86.15-96.69) | 0.593 | 262.00 | 75.00 | 94.09 | 71.05 | 95.11 |
| HE4 | 93.14 | (88.64-97.64) | 0.965 | 114.00 | 86.11 | 87.63 | 57.41 | 97.02 |
| ROMA | 94.00 | (89.75-98.25) | 0.738 | 53.17 | 86.11 | 90.32 | 63.27 | 97.11 |
| Sassone | 78.91 | (70.55-87.27) | 0.001\* | 10.00 | 66.67 | 82.80 | 42.86 | 92.77 |

Abbreviation: ROC = Receiver operating characteristic, AUC = Area under the curve, CI = Confident interval, Se = Sensitivity, Sp = Specificity, PPV = Positive predictive value, NPV = Negative predictive value, RMI = Risk of Malignancy Index, CA125 = Carcinoma antigen 125, HE4 = Human epidermis protein 4, ROMA = Risk of Ovarian Malignancy Algorithm, EOC = Epithelial ovarian cancer

\*Significant at p < 0.05

**Figure 2** Receiver operating characteristic plot and area under curve of Risk of Malignancy Index, CA-125, HE4, Risk of Ovarian Malignancy Algorithm and Sassone score for predicting ovarian malignancy in patients



Abbreviation: RMI = Risk of Malignancy Index, CA-125 = Carcinoma antigen 125, HE4 = Human epidermis protein 4, ROMA = Risk of Ovarian Malignancy Algorithm, ROC = Receiver operating characteristic

The performance of all five tests in distinction between patients with non-cancer conditions and ovarian cancers stratified by menopausal status was demonstrated in Table 6. In premenopausal women, RMI, ROMA and HE4 level showed the greatest areas under ROC curves (85.57%, 84.36% and 84.37%, respectively), whereas Sassone score showed the least AUC (73.08%). In contrary, HE4 level revealed the least area under ROC curve (77.08%) in postmenopausal women but RMI value still presented the greatest AUC (87.85%). At optimal cutoff value, HE4 level and ROMA value had the highest sensitivity (85.71% and 82.14%, respectively) and NPV (96.04% and 95.10%, respectively) in premenopausal women, while RMI value had the highest sensitivity (89.13%) and NPV (91.07%) in postmenopausal women.

**Table 6** Comparison of the performance of Risk of Malignancy index (RMI), CA125, HE4, Risk of Ovarian Malignancy Algorithm (ROMA) and Sassone score for prediction of ovarian cancer stratified by menopausal status

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patients (N) | Tests | ROC | | | Optimal cutoff value | Se (%) | Sp (%) | PPV (%) | NPV (%) |
| AUC | (95%CI) | p |
| Premenopause  Non-cancer (120) vs. Cancer (28) | RMI | 85.57 | (76.93-94.21) |  | 300.00 | 74.43 | 87.50 | 57.14 | 92.92 |
| CA125 | 81.70 | (71.38-92.01) | 0.341 | 245.50 | 60.71 | 92.50 | 63.38 | 90.98 |
| HE4 | 84.36 | (74.45-94.27) | 0.816 | 65.00 | 85.71 | 80.83 | 51.06 | 96.04 |
| ROMA | 84.37 | (74.43-94.32) | 0.813 | 14.18 | 85.71 | 80.83 | 50.00 | 95.10 |
| Sassone | 73.08 | (63.50-82.64) | 0.031\* | 10.00 | 57.14 | 81.67 | 42.11 | 89.09 |
| Postmenopause  Non-cancer (66) vs. Cancer (46) | RMI | 87.85 | (81.34-94.35) |  | 169.11 | 89.13 | 77.27 | 73.21 | 91.07 |
| CA125 | 83.51 | (75.59-91.44) | 0.052 | 58.37 | 78.26 | 78.79 | 71.43 | 82.54 |
| HE4 | 77.08 | (67.61-86.54) | 0.023\* | 134.90 | 63.04 | 83.33 | 71.79 | 75.34 |
| ROMA | 83.99 | (76.24-91.74) | 0.143 | 44.29 | 78.26 | 80.30 | 73.47 | 84.13 |
| Sassone | 80.39 | (72.09-88.69) | 0.119 | 10.00 | 67.39 | 84.85 | 75.61 | 78.87 |

Abbreviation: ROC = Receiver operating characteristic, AUC = Area under the curve, CI = Confident interval, Se = Sensitivity, Sp = Specificity, RMI = Risk of Malignancy Index, CA-125 = Carcinoma antigen 125, HE4 = Human epidermis protein 4, ROMA = Risk of Ovarian Malignancy Algorithm

\*Significant at p < 0.05

The detail of false positive and false negative cases based on the standard cutoff level criteria of RMI, CA125, HE4, ROMA and Sassone US score according to their histopathology type were summarized in table 7. Patients with endometriotic cysts revealed many amount of false negative cases, especially the prediction by ROMA value (41.67%). Moreover, ROMA value yielded the greatest number of false positive cases in patients with non-cancer diseases (31.18%). In case of ovarian cancer, patients with clear cell carcinoma and non-EOCs had great number of false negative cases in all 5 parameters. In addition, HE4 level showed the greatest number of false negative cases (33.78%).

**Table 7** Numbers of false positive and false negative cases of each parameter at standard cutoff values

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Pathologic diagnosis | | | N | RMI  (%) | | CA125 (%) | | HE4  (%) | | ROMA (%) | | Sassone (%) | |
| False positive in non-cancer | | | | | | | | | | | | | |
|  | Endometriotic cyst | | 48 | 14 | (29.17) | 13 | (28.08) | 6 | (12.50) | 20 | (41.67) | 9 | (18.75) |
|  | Teratoma | | 30 | 2 | (6.67) | 2 | (6.67) | 2 | (6.67) | 2 | (6.67) | 8 | (26.67) |
|  | Serous cystadenoma | | 21 | 2 | (9.52) | 1 | (4.76) | 4 | (19.05) | 4 | (19.05) | 4 | (19.05) |
|  | Mucinous cystadenoma | | 31 | 6 | (19.35) | 5 | (16.13) | 3 | (9.68) | 9 | (29.03) | 8 | (25.81) |
|  | Functional/Simple/Paratubal cyst | | 7 | 3 | (42.56) | 3 | (42.86) | 2 | (28.57) | 3 | (42.86) | 3 | (42.86) |
|  | Tuboovarian abscess | | 7 | 3 | (42.86) | 1 | (14.29) | 3 | (42.86) | 3 | (42.86) | 3 | (42.86) |
|  | Leiomyoma | | 7 | 1 | (14.29) | 1 | (14.29) | 0 | (0.00) | 1 | (14.29) | 6 | (85.71) |
|  | Fibrothecoma | | 3 | 0 | (0.00) | 0 | (0.00) | 0 | (0.00) | 0 | (0.00) | 1 | (33.33) |
|  | Brenner tumor | | 2 | 1 | (50.00) | 1 | (5.00) | 0 | (0.00) | 1 | (50.00) | 0 | (0.00) |
|  | Pseudocyst | | 2 | 0 | (0.00) | 0 | (0.00) | 1 | (50.00) | 2 | (100.00) | 0 | (0.00) |
|  | Borderline tumor | | 28 | 6 | (21.43) | 8 | (28.57) | 5 | (17.86) | 13 | (46.43) | 13 | (46.43) |
|  | Total | | 186 | 38 | (20.43) | 35 | (18.82) | 26 | (13.98) | 58 | (31.18) | 55 | (29.57) |
| False negative in ovarian cancer | | | | | | | | | | | | | |
|  | EOC | | 66 | 13 | (19.70) | 17 | (25.76) | 20 | (30.30) | 8 | (12.12) | 22 | (33.33) |
|  |  | Serous carcinoma | 13 | 1 | (7.69) | 2 | (15.38) | 2 | (15.38) | 1 | (7.69) | 3 | (25.76) |
|  |  | Mucinous carcinoma | 8 | 1 | (12.50) | 3 | (37.50) | 5 | (62.70) | 2 | (25.00) | 4 | (23.08) |
|  |  | Endometrioid carcinoma | 14 | 2 | (14.29) | 2 | (14.29) | 1 | (7.14) | 0 | (0.00) | 5 | (35.71) |
|  |  | Clear cell carcinoma | 19 | 7 | (36.84) | 8 | (42.11) | 10 | (52.63) | 5 | (26.32) | 6 | (31.58) |
|  |  | Mixed EOC | 8 | 2 | (25.00) | 2 | (25.00) | 2 | (25.00) | 0 | (0.00) | 3 | (37.50) |
|  |  | Adenocarcinoma, NOS | 4 | 0 | (0.00) | 0 | (0.00) | 0 | (0.00) | 0 | (0.00) | 1 | (25.00) |
|  | Non-EOC | | 8 | 3 | (37.50) | 3 | (37.5) | 5 | (62.50) | 4 | (50.00) | 1 | (12.50) |
|  | Total | | 74 | 16 | (21.62) | 20 | (27.03) | 25 | (33.78) | 12 | (16.22) | 23 | (31.08) |

Abbreviation: RMI = Risk of Malignancy Index, CA-125 = Carcinoma antigen 125, HE4 = Human epidermis protein 4, ROMA = Risk of Ovarian Malignancy Algorithm, EOC = Epithelial ovarian carcinoma, NOS = not otherwise specific

**DISCUSSION**

Discrimination between benign and malignant ovarian tumors is essential for creating an optimize system for patient referrals without overburdening reference centers. Benign pelvic masses can be treated locally, while treatment of patients suspected of ovarian cancer should take place at a referral center by gynecologic oncologists. Studies have shown that ovarian cancer patients who are managed in specialized centers by gynecologic oncologists have improved outcome ([21](#_ENREF_21)).

From the results of the present study, the RMI value had the best performance in differentiating non-cancer diseases and ovarian cancer. Nevertheless, the AUC of ROC curve for the RMI value is slightly higher than for the ROMA value. Both RMI and ROMA values had an advantage over CA125 level and Sassone score in the prediction of ovarian cancer in the presence of a pelvic mass. Both RMI and ROMA values had the high accuracy in discrimination of both early and advanced stage ovarian cancer from non-cancer diseases, while the CA125 level showed the lowest accuracy in those with early stage ovarian cancer. In premenopausal women, all of the RMI, ROMA and HE4 level revealed the highest accuracy, whereas only the RMI level had the highest accuracy in postmenopausal women but the HE4 level appeared in the lowest accuracy.

Jacobs et al ([10](#_ENREF_10)) created the RMI I using the cutoff value of 200 shown to be associated with a higher risk of malignancy and demonstrated a sensitivity of 85.4% and a specificity of 96.9%. Two prospective multicenter studies including 548 and 1159 patients showed for RMI a sensitivity of 81% and 92% and a specificity of 85% and 82% respectively at a cutoff value of 200 ([22](#_ENREF_22), [23](#_ENREF_23)). A systematic review of 83 predictive models (including RMI and Sassone score) for ovarian malignancy published in 2009 ([24](#_ENREF_24)) suggested that RMI with a pooled sensitivity of 78% and specificity of 87% should be the prediction model of choice in pre-operative assessment of an adnexal mass. The sensitivity and specificity of RMI as shown in this study (83.78% and 78.49%) were comparable with these previous studies.

HE4 was a recently discovered biomarker that had been shown to have an AUC 90.8%, sensitivity of 72.9% at set of 95% specificity for differentiating between benign ovarian diseases and ovarian cancers, and these values were higher than those related to the use of CA125 (AUC 83.6% and sensitivity 43.3%) and the significantly increased performance was found when combining HE4 and CA125 (area under ROC 91.4% and sensitivity 76.4%) ([12](#_ENREF_12)). In 2009, Moore et al ([25](#_ENREF_25)) proposed that the ROMA, the dual marker algorithm utilizing serum HE4 and CA125, could be applied to evaluate ovarian tumors resulted in a sensitivity of 88.7% at a specificity of 74.7%. However, the study by Van Gorp et al ([26](#_ENREF_26)) evaluating pelvic mass patients illustrated that HE4 and ROMA did not supply significantly in the differentiation of ovarian cancer from benign pelvic masses compared to CA125 alone (AUC 89.9%, 85.7% and 87.7%, respectively). From the present study, AUC for ROMA performed better than for HE4 and CA125 (AUC 86.16%, 82.36% and 80.59%, respectively).

Chan et al ([27](#_ENREF_27)) studied the performance of HE4 and ROMA for distinguishing between benign and malignant pelvic masses in Asian women found that ROMA, HE4 and CA 125 had areas under ROC of 95%, 93% and 89%, respectively, which was greater than those demonstrated by this study.

In the later study by Moore et al ([18](#_ENREF_18)), ROMA was compared to RMI for prediction of ovarian cancer in patients with a pelvic mass. ROMA had a significantly greater area under AUC of 95.3% compared to RMI with an area under AUC of 87.0%. The present study results, found RMI performed slightly better than ROMA (AUC 87.61% vs. 86.16%) in discriminating benign pelvic masses and ovarian cancer, were not in agreement with the result by Moore et al ([18](#_ENREF_18)). The present study’s results were consistent with study by Karlsen et al ([28](#_ENREF_28)) which found RMI performed slightly better than ROMA (AUC 95.8% vs. 95.4%).

Sassone score devised by Sassone et al ([7](#_ENREF_7)) was the most evaluated prediction model and had the pooled sensitivity and specificity of 84% and 80% from systematic review ([24](#_ENREF_24)) which those were higher than the sensitivity (65.31%) and specificity (82.80%) in the present study. US examination has some limitation since this depends on the operators and is relatively subjective assessment. The major weakness of the mutiparametric US scoring systems are that they was developed in its respective institutional centers, and when they were externally validated in a new population, they did not perform as well. The differences in these data are likely due to differences in interpretation of US findings and variation between patient populations.

In the present study, HE4 showed the least performance in postmenopausal women. Several studies have described HE4 as a potential better marker compared to CA125 in the diagnostic process of premenopausal women with a pelvic mass ([12](#_ENREF_12), [18](#_ENREF_18), [27](#_ENREF_27)). Serum HE4 concentrations vary significantly on the basis of age and are tendency to be elevated in advancing age ([29](#_ENREF_29)). The higher baseline HE4 level in the older age group would reduce the gap between non-cancer and cancer categories, thus reducing in the performance of test.

The major problem associated with the pre-surgery evaluation of pelvic masses concerns the diagnosis of endometriosis, borderline tumor, clear cell carcinoma and non-EOCs. The present study found that all five methods performed the high number of false negative cases in clear cell carcinoma and non-EOCs. Therefore, those are a defect of all five parameters and further improvement of novel biomarkers in differentiating pelvic masses are still needed. In case of endometriotic patients, 41.67% of cases had higher ROMA value than the standard cutoff but only 12.5% had higher HE4 level. The increased specificity of HE4 suggests that the use of these parameters can improve the evaluation.

Borderline tumors have the problematic approach in classification to be non-cancer or cancer categories. In Asian women, the borderline tumors are reported in the higher incidence and have a variation in histopathologic subtypes, especially mucinous borderline tumor ([30](#_ENREF_30)). The authors found that all five parameters demonstrated better performance when borderline tumors were classified as non-cancer category. Although historical recommendation that patients with a borderline tumor should undergoing surgical staging at the time of their initial surgery, patients with a borderline tumor on final pathology receive little benefit from comprehensive surgical staging and do not require adjuvant treatment. Clinical and biological evidence suggests that these tumors can be classified as low-risk and has a good prognosis. Therefore, their inclusion in the non-cancer category appears to be acceptable.

This prospective cohort study compared the common predictive parameters (RMI, CA125 and Sassone score) with the new parameters (HE4 and ROMA) in Asian women. Most of the previously published data are based on Caucasian women. The measurement bias was limited by using only one ultrasonographic operator and one ultrasonographic machine, accompanied with blinding the biomarker levels.

The limitation of the present study is that it is a hospital-based prospective cohort from a referral hospital, thus it may not be representative of the whole country. Concerning the benefit of these parameters, there are several conflicting studies which some studies reporting that ROMA and HE4 has the better prediction of ovarian malignancy than RMI and CA125 ([14](#_ENREF_14), [17](#_ENREF_17), [18](#_ENREF_18)) and other studies showing no benefit ([26](#_ENREF_26), [28](#_ENREF_28)). This could be due to the difference in the study population. Asian women tend to have the higher incidence of mucinous borderline tumor and clear cell carcinoma compared to Caucasian women, impact on the different performance of tests, especially CA125, HE4 and ROMA.

Although the ROMA and RMI is not significant difference in accuracy, ultrasound dependency of RMI is major obstacle to the validity and accuracy of test due to the success of ultrasound assessment depends on the sonographer’s expertise. Overall, ROMA might be valuable as a first-line biomarker for assess risk for women who present with pelvic masses for optimize referral to tertiary center. However, the cost-effectiveness of ROMA compared with RMI for differentiation between benign diseases and ovarian cancer must be considered.

In summary, RMI was the most numerically accurate method but ROMA had not significantly different in accuracy with RMI in discriminating between benign ovarian diseases and ovarian cancer. However, clear cell carcinoma and non-EOCs were the defect of all five parameters.

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**Conflict of interest statement**

There are no conflicts of interest in this study.

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