A novel biomarker panel of HE4 and D-dimer improves ovarian cancer diagnosis in postmenopausal women presenting with symptomatic pelvic masses: a prospective cohort study

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Abstract

Objective: Cancer Antigen 125 (CA125), the biomarker in common clinical use for ovarian cancer, is limited by low sensitivity for early disease and high false positives. The aim of this study was to evaluate several candidate biomarkers, alone or in combination, compared to CA125. Design: A prospective observational cohort study Setting: St. James's Hospital (SJH), a tertiary referral centre for gynaecological malignancy in Dublin, Ireland. Population: 274 patients undergoing surgery for symptomatic pelvic masses between 2012 and 2018. Methods: Preoperative Human Epididymis Protein 4 (HE4), the Risk of Ovarian Malignancy Algorithm, the Risk of Malignancy Index I and II, D-dimer, and fibrinogen were assessed. Logistic regression models were fitted for each biomarker alone and in combination. AUCs and pAUCs in the 90-100% specificity range were determined. Main Outcome measures: The accuracy of biomaker(s) in the prediction of malignant/borderline versus benign tumour status compared to CA125. Results: 89 pre- and 185 post-menopausal women were included. In premenopausal women, no biomarker(s) outperformed CA125 (AUC 0.73; 95% CI 0.63-0.84). In postmenopausal women, HE4 had a pAUC of 0.71 (95% CI 0.64-0.79) compared with 0.57 (95% CI 0.51-0.69) for CA125 ($p = 0.009$). HE4 + D-dimer had an improved pAUC of 0.74 (95% CI 0.68-0.81, $p < 0.001$). Conclusion: A novel biomarker panel of HE4 + D-dimer outperformed CA125 alone as a high specificity biomarker in postmenopausal women and could aid in the preoperative triaging of symptomatic pelvic masses.

Introduction

Approximately 1.3% of women are diagnosed with ovarian cancer (OC) over their lifetime and less than half of these survive 5 years following diagnosis (1). OC symptoms are difficult to differentiate from those of benign and other conditions (2, 3). Resultantly, the majority of cases are metastatic at diagnosis (4). An estimated 10% of pre- and 20% of post-menopausal women presenting with a pelvic mass ultimately receive a diagnosis of OC (5). The challenge for clinicians is to identify and refer those cases most suspicious for OC to a specialist gynaecologic oncologist at the earliest possible stage of the disease. This intervention has been shown to improve prognosis (6-8). Effective triaging would enable lower risk cases to undergo less invasive surgery, possibly with preservation of fertility, and to remain in the care of general gynaecology services.

Current work-up of a pelvic mass centers on CA125 and transvaginal ultrasound (TVUS), both of which have limitations. CA125 is elevated in benign gynaecologic conditions (9), increased in only 50% of early OC (1, 10), and not expressed in 20% of OC (11). While TVUS frequently detects ovarian cysts, most are benign and accurate diagnosis is user-dependent (12, 13). Formal ultrasound-based criteria increase sensitivity but are less specific than subjective US testing (14).

Several tumour biomarkers have been identified to improve risk-stratification. Human Epididymis Protein 4 (HE4) has shown the most promise (15). It is found in particularly high concentrations in serous and endometrioid OC, and detected in up to 50% of cases where CA125 is not (16). HE4 is elevated in earlier stages (FIGO I-II) (16) and less affected by benign conditions than CA125 (17). Based on encouraging initial studies (18), HE4 was FDA-approved in 2011 combined with CA125 and menopausal status in the Risk of Ovarian Malignancy Algorithm (ROMA) (19). However, subsequent studies yielded conflicting results (20, 21).

Other algorithms have been formulated to improve on the above biomarkers but no clear conclusions of clinical benefit have been drawn due to a lack of prospective trials (22, 23). Amongst them are the combinations of CA125 + HE4 (24), and CA125 + D-dimer (25). The Risk of Malignancy Index (RMI) I or II (26) are weighted products of CA125, menopausal score and ultrasound score. The addition of fibrinogen to this combination has also been described (27). In the past decade, over 50 novel biomarkers have been reported to improve on the sensitivity of CA125 (11, 28-30)

Given the uncertainty regarding the optimal risk-stratification method for pelvic masses, we sought to evaluate HE4, ROMA, RMI I, RMI II, D-dimer, and fibrinogen, alone or in combination, compared with CA125.

Methods

Study Population

This was a prospective cohort study performed at St. James's Hospital (SJH), a tertiary referral centre for gynaecological malignancy in Dublin, Ireland. Patients undergoing surgery for a pelvic mass were invited to participate and donate pre-operative blood samples and health data to the DISCOVARY bioresource. Sequential patients presenting with a symptomatic pelvic mass over a 6-year period between 2012 and 2018 and who underwent surgery were included in this study. All cases were suspected to be ovarian malignancies based on MDT discussion. Prior to recruitment, all patients provided written, informed consent and the study was approved by the SJH Research Ethics Committee. Tumours that were not ovarian in origin on final histology, representing either metastases to the ovary or disease at another site entirely, were included in the final study analysis as well as benign and borderline/malignant ovarian cases. Recurrent OC, patients undergoing neoadjuvant chemotherapy and patients presenting with venous thromboembolism were excluded.

Tumour status (benign, borderline or malignant), menopausal status and age were recorded for all patients. Menopause was defined as the absence of menstruation for 12 consecutive months and/or age 50 years with a history of previous hysterectomy. Pre-operative laboratory values of CA125, HE4, D-dimer, and fibrinogen and RMI ultrasound features were recorded.

Laboratory Methods

Pre-operative serum samples were centrifuged, snap frozen with liquid nitrogen and stored at -80@C until analysed. Serum biomarker levels were analysed for CA125 and HE4 using the Roche e602 Immunoassay platform. Matched plasma samples were used to measure D-dimer levels using the Vidas(r) D-Dimer Exclusion(tm) II assay and fibrinogen levels using the Clauss method (31).

Calculation of ROMA

A Predictive Index (PI) was calculated for premenopausal and postmenopausal patients separately using equations (1) and (2) below. The HE4 and CA125 results were inserted into the appropriate equation:

Premenopausal:

Predictive Index (PI) = -12.0 + 2.38*LN [HE4] + 0.0626*LN [CA125]

Postmenopausal:

Predictive Index (PI) = -8.09 + 1.04*LN [HE4] + 0.732*LN [CA125]

To calculate the ROMA value, the calculated value for Predictive Index was inserted into equation (3):

ROMA value % = exp (PI) / [1 + exp (PI)] *100

Calculation of RMI I and II

The RMI was calculated as first described by Jacobs *et al* .(26) as a product of pre-operative CA125 level in U/ml, menopausal status, and ultrasound (US) score. Premenopausal status gave 1 mark for both RMI I and II while postmenopausal status gave 2 and 4 marks, respectively, for RMI I and II. US reports were reviewed, and 1 mark given to each of 5 features (multilocular cyst, solid areas, metastases, ascites and bilateral lesions). The US score was then assigned for RMI I as follows:

U = 0 for ultrasound score of 0

U = 1 for ultrasound score of 1

U = 3 for ultrasound score of 2 – 5.

And for RMI II:

U = 1 for ultrasound score of 0 or 1

U = 4 for ultrasound score of 2 or more.

Statistical Analysis

As biomarker distributions were not normally distributed based on the Shapiro-Wilk test (<0.05), median values were compared using the Wilcoxon rank sum test. A p-value of <0.05 was deemed to be significant.

Cases were analysed in two cohorts: pre- and post-menopausal. The outcomes were dichotomised into benign or borderline/malignant for all statistical analyses. Biomarkers of interest and combinations of these biomarkers were used as predictors for both cohorts.

Sensitivity and specificity were calculated for the following biomarkers using the following a priori cut-off values in parentheses: CA125 (35 U/ml), HE4 (70pM/L in premenopausal women and 140 pM/L in post-menopausal women), ROMA ([?]11.4% in premenopausal women and [?]29.9% in postmenopausal women), RMI I (250), RMI II (250), fibrinogen (3.5 g/L) and D-dimer (500 ng/ml).

Biomarkers were right-skewed and were thus log-transformed before logistic regression analysis. Combination panels were constructed by entering biomarkers into a binary logistic regression model with a dichotomous outcome of benign or borderline/malignant. Receiving-Operating Characteristic (ROC) curves and their corresponding 95% CI were determined for each biomarker and combination panel. Overall assessment of predictor performance was evaluated using the ROC Area Under the Curve (AUC). Each predictor's AUC was compared against that of CA125, the most commonly used biomarker for detection of OC in clinical practice, using the method of DeLong et al (32). Optimal cutoffs for each biomarker are shown using the minimum distance from the top-left of the ROC curve. This is a method of providing the best balance between sensitivity and specificity for a predictor (33).

As it is desirable to have a high specificity biomarker, the partial area under the curve (pAUC) of each panel's ROC curve was determined in the region of the curve where for the specificity ranges from 90-100%. Confidence intervals and comparisons of pAUCs for panels against CA125 were calculated using bootstrapping with 10,000 repeats as previously described (34). Biomarker cutoffs are shown based on the top-left method within the high specificity range and at 98% specificity with the corresponding sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) at that cutoff. All statistical analyses were performed in R Version 3.53 (R Foundation). AUC and pAUC statistics were calculated using the pROC package (35).

Results

Patient demographics

274 sequential patients from the DISCOVARY bioresource met the inclusion criteria and were included in the study, of whom 89 were pre- and 185 post-menopausal. Patient demographics are displayed in **Table 1** . The premenopausal group consisted of 47 benign, 21 borderline, and 21 malignant cases while the postmenopausal group had 97 benign, 20 borderline and 68 malignant tumours.

Of the malignant cases, 12 were non-ovarian in origin and a further 7 cases consisted of mixed ovarian and non-ovarian histology. Serous was the most common malignant subtype (47.2%). Ovarian malignancies were most commonly high grade (54%). The distribution of cancer stages was stage 1 (29.2%), stage 2 (4.5%), stage 3 (29.2%), and stage 4 (18%) (Table 1).

Biomarker performance

Median levels of CA125, HE4, D-dimer, fibrinogen, ROMA, RMI I, and RMI II were all significantly higher in borderline/ malignant than in benign cases ($p < 0.001$) (**Table S1** and **Figure S1**). Pre-operative US scoring for RMI calculation was not possible in a significant number of patients as pre-operative US reports were not available.

Table 2 demonstrates the AUC-ROC for each biomarker/ combination of biomarkers, analysed as continuous variables, for premenopausal and postmenopausal groups respectively.

Premenopausal Group

In the premenopausal group, CA125 had an AUC of 0.73 (95% CI 0.63-0.84) (**Table 2A**). Two panels, (i) HE4 + Fibrinogen and (ii) HE4 + D-dimer + Fibrinogen, had ROC-AUCs of 0.76 (95% CI 0.64-0.87). However, no biomarker or biomarker panel was significantly different when compared to CA125 in premenopausal women.

Postmenopausal Group

In the postmenopausal group, HE4 had an AUC of 0.81 (95% CI 0.74-0.87) but was not statistically significant compared to CA125 (AUC 0.77; 95% CI 0.71-0.84; $p = 0.371$) (**Table 2B**). HE4 had a specificity of 84.5% at a sensitivity of 70.5%. The addition of D-dimer or Fibrinogen to the model resulted in AUCs of 0.83 (95% CI 0.77-0.89) and 0.81 (95% CI 0.74-0.88), respectively.

Using the pre-defined cutoff values described above, sensitivity and specificity levels for all biomarkers were calculated and are displayed in **Table S2.** HE4 had a specificity of 92%.

Optimal Biomarker Cutoffs and partial AUCs

As a high specificity biomarker is desirable, partial AUCs for biomarkers and combination panels for the high specificity region of 90-100% were constructed for the postmenopausal group and are shown in**Table 3** and **Figure 1** . HE4 had a corrected pAUC of 0.71 (95%CI 0.64-0.79) compared to a pAUC of 0.57 (95% CI 0.51-0.69) for CA125 ($p = 0.009$). This was followed by D-Dimer (pAUC 0.66, 95% CI 0.6-0.74) and ROMA (pAUC 0.68; 95% CI 0.62-0.76). At 98% specificity, HE4 had a sensitivity of 44.3% compared to 9.1% for CA125 ($p = 0.009$). HE4 had a specificity of 90.7% and a sensitivity of 58% at a cut-off of 111.75 pm/L.

A combination of HE4 and D-dimer had a corrected pAUC of 0.74 (95%CI 0.68-0.81) compared with 0.57 for CA125 (95%CI 0.51-0.69, $p < 0.001$). The addition of CA125 ± Fibrinogen provided only modest improvement in the pAUC to 0.75 (95% CI 0.68-0.82, $p < 0.001$). Optimal cut-off values for each predictor are displayed for the 90-100% and 98% specificity regions.

Validation Sample

A separate validation sample was selected from the DISCOVARY bioresource (**Table S3**) and used to calculate the probability of malignancy for selected models derived from the original test sample (**Table S4**). This consisted of preoperative blood samples collected from 133 postmenopausual women between 2006 and 2012, with the same inclusion and exclusion criteria applied to the main study cohort. This cohort was limited as D-dimer and RMI biomarkers were unavailable.

HE4 had an AUC of 0.8 (95% CI 0.72-0.88) compared to an AUC of 0.75 for CA125 (95% CI 0.66-0.83; p = 0.181). The addition of Fibrinogen ± CA125 to HE4 resulted in a modest increased in the AUC to 0.82 (95% CI 0.73-0.91, p = 0.058) and 0.83 (95% CI 0.74-0.92, p = 0.193), respectively. As D-dimer and RMI scores were not available in the validation sample, it was not possible to assess any models that included D-dimer or to perform a ROC comparison analysis.

Discussion

Main Findings

This study showed that among postmenopausal patients, HE4 significantly outperformed CA125 as a high specificity biomarker with a pAUC of 0.71 (95% CI 0.64-0.79) compared with 0.57 (95% CI 0.51-0.69) for CA125 (p = 0.009). A model combining HE4 + D-dimer improved the pAUC to 0.74 (95% CI 0.68-0.81) versus CA125 (p < 0.001). This is, to our knowledge, the first study to assess the diagnostic accuracy of this combination of biomarkers. No panel statistically outperformed CA125 in the premenopausal group of this study.

Strengths and limitations

The strengths of this study include its relatively large study population, choice of widely available biomarkers and pragmatic inclusion criteria. Differences in laboratory assays, threshold values and case definitions have contributed to a wide disparity in the performance of novel biomarkers. For example, the retrospective exclusion of borderline and non-epithelial OC has been shown to increase the sensitivity of ROMA (37). In order to reflect clinical practice, this study included borderline and non-ovarian tumours as well as all subtypes of OC in the primary outcome for two reasons. Firstly, a pelvic mass encompasses a wide range of differentials which would be unknown at the time of presentation. Secondly, borderline tumours routinely undergo at least intraperitoneal staging surgery. Furthermore, stratification of women by menopausal status minimised variablility seen with CA125 and HE4, in particular, with age and physiological status.

This study has some limitations. It was performed in a single institution and, as a tertiary gynaecologic referral centre, the women in this study represented an older, higher risk population compared to the general population where this panel would be intended for use. For example, a proportion of these women would have been referred as urgent cases with CA125 levels of >200kU/ml in accordance with the national guidelines (38). This may lead to overestimation of the values of HE4, D-dimer, and fibrinogen, which have been shown to correlate with advanced stage and poorer prognosis.

The performance of other biomarkers in this study, in particular RMI and ROMA, could be underestimated as they were not intended for use in a tertiary centre (39). Assessment of RMI was further limited by a lack of strict RMI protocols used in ultrasound reports (40). To account for this selection bias, the study would need to be expanded to the primary care setting or to a hospital with general gynaecology services as a first step. Alternatively, increased cutoffs could be applied for a high-risk population as were created in this study but this would need to be validated in other tertiary referral centres.

Finally, the validation was limited to a relatively small population and a larger population is needed to confirm the findings. Although the combination of HE4 + fibrinogen performed well with an AUC-ROC of 0.82 in a separate cohort of postmenopausal women, D-dimers were not available for this cohort and the panel could not be fully validated.

Interpretation

It is estimated that 10% of women will undergo surgery for a pelvic mass during their lifetime (41). There is currently no clinical algorithm in routine use for the preoperative triaging of such masses. Effective triaging would direct patients to the optimal surgical pathway.

Several algorithms have been proposed for use in the assessment of pelvic masses, two of which are FDA-approved. ROMA uses a model derived from CA125, HE4 and menopausal status to classify women presenting with a pelvic mass as either high or low risk for OC. It was initially validated in a multicentre clinical trial

of 472 postmenopausal women presenting with pelvic masses. In this study, it demonstrated an impressive sensitivity and specificity of 92.3% and 76.0%, respectively, for the detection of OC (18). However, several subsequent studies have yielded conflicting results. For example, a prospective study of 389 women showed no added benefit of ROMA (AUC-ROC 0.898) over CA125 alone (AUC-ROC 0.877) (20). A recent cohort study of 1590 women with pelvic masses found that HE4 added no value to CA125 and TVUS (21).

The second FDA-approved algorithm is the OVA1 test, a multivariate assay which includes CA-125, transthyretin, apolipoprotein A1, ß2-microglobulin, transferrin and transthyretin. In a prospective, multicentre trial of 494 women with pelvic masses, it demonstrated higher sensitivity (95.7%, 95%CI 89.3–98.3) and negative predictive value (98.1%, 95%CI 95.2–99.2) for OC compared to CA125-II, a second generation CA125 assay (42). A smaller study of 118 women found no added benefit for this panel (43).

Our proposed model of HE4 + D-dimer for the postmenopausal group compared favourably to the current FDA-approved algorithms. HE4 + D-dimer had an AUC of 0.83 (95% CI 0.77-0.89) compared to an AUC of 0.78 for ROMA (95% CI 0.71-0.85). A potential advantage of our panel compared to OVA1 or panels that include radiological imaging is that D-dimer, in particular, is relatively inexpensive to perform, routinely measured in clinical practice, and operator-independent.

The AUC-ROC is a useful measure of overall diagnostic performance. However, it includes regions which are of little clinical relevance such as those with low specificity. (44). Sensitivities of >75% and a remarkably high specificity of 99.6% have been proposed for the screening of asymptomatic women for OC in order to achieve a high positive predictive value and minimize false positive results (11). For the postmenopausal group of this symptomatic study population, a slightly broader predefined specificity of 90-100% was applied for pAUC calculation. At this range, HE4 + D-Dimer had a corrected pAUC of 74.22 (95% CI 67.88-81.16) with a sensitivity of 62.5%. HE4 was the best performing single biomarker (pAUC 71.21, 95% CI; 63.93-79.01) and significantly outperformed CA125 ($p = 0.009$). The addition of fibrinogen to HE4 + D-dimer was associated with only a modest improvement in pAUC.

Fibrinogen and its end product D-dimer have been shown to predict poor prognosis in OC, independent of venous thromboembolism (45-48). Their use as potential diagnostic biomarkers, however, is often overlooked by researchers as alterations in their levels can be seen in a wide range of conditions (49). They have not previously been studied alongside HE4 for OC diagnosis. However, a similar panel has recently been described for the diagnosis of endometrial cancer (EC) (50). In a study of 254 women, a panel combining HE4 + D-dimer + fibrinogen + CA199 was the best predictor of EC with a sensitivity and specificity of 91.34% and 70.08%, respectively, at the optimal cut-off value. This supports the diagnostic potential of serum D-dimer and fibrinogen, something which few previous studies have examined.

Conclusion

The addition of biomarkers to CA125 does not increase ovarian cancer detection in premenopausal women. HE4 alone and in combination with D-dimer had a high specificity and may play a role as a second-line test in the setting of premenopausal women with an inconclusive TVUS and a positive CA125.

Our results showed that among postmenopausal patients, HE4 significantly outperformed CA125 as a high specificity biomarker. The combination of HE4 + D-dimer improves on the diagnostic accuracy of CA125 alone and could help with the preoperative assessment of pelvic masses. This study also proposed new biomarker cutoffs in this symptomatic population. Prospective studies are required to validate the performance of this panel and to assess the cost efficacy of adding additional analytes to the current diagnostic work-up.

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Disclosure of Interests

All authors declare no conflicts-of-interests related to this article.

Contribution to Authorship

SOT and KM planned and designed the study. NG and FS recruited patients to the study. KM, YH, LN and SOT collected patient samples. AS and MR performed HE4 assays. SD performed the data analysis. KM wrote the first draft of the article. SD, YH, AS, MR, NG, FS, JOL, LN, and SOT revised the manuscript and approved the final version for publication.

Details of Ethics Approval

The study was approved by the St. James's Hospital and the Adelaide Meath and National Children's Hospital research ethics committee (biobank approval 2004, 041213/12904 and subsequent amendments in 2009 (Ref 2009/29/01) and 2014 (Ref. 2014-10 List 11)). All included patients provided written informed consent for participation.

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References

1. National Cancer Institute NIoH. Cancer stat facts: ovarian cancer 2018 [Available from: https://seer.cancer.gov/statfacts/html/ovary.html.

2. Jacobs IJ, Menon U. Progress and challenges in screening for early detection of ovarian cancer. Molecular & Cellular Proteomics. 2004;3(4):355-66.

3. Goff BA, Mandel L, Muntz HG, Melancon CH. Ovarian carcinoma diagnosis. Cancer. 2000;89(10):2068-75.

4. Li J, Dowdy S, Tipton T, Podratz K, Lu W-G, Xie X, et al. HE4 as a biomarker for ovarian and endometrial cancer management. 2009.

5. Bast RC, Jr., Skates S, Lokshin A, Moore RG. Differential diagnosis of a pelvic mass: improved algorithms and novel biomarkers. Int J Gynecol Cancer. 2012;22 Suppl 1:S5-8.

6. Bristow RE, Zahurak ML, Diaz-Montes TP, Giuntoli RL, Armstrong DK. Impact of surgeon and hospital ovarian cancer surgical case volume on in-hospital mortality and related short-term outcomes. Gynecologic oncology. 2009;115(3):334-8.

7. Mayer AR, Chambers SK, Graves E, Holm C, Tseng PC, Nelson BE, et al. Ovarian cancer staging: does it require a gynecologic oncologist? Gynecologic oncology. 1992;47(2):223-7.

8. Havrilesky LJ, Whitehead CM, Rubatt JM, Cheek RL, Groelke J, He Q, et al. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. Gynecol Oncol. 2008;110(3):374-82.

9. Markman M. The Role of CA-125 in the Management of Ovarian Cancer. Oncologist. 1997;2(1):6-9.

10. Urban N, McIntosh MW, Andersen M, Karlan BY. Ovarian cancer screening. Hematol Oncol Clin North Am. 2003;17(4):989-1005, ix.

11. Yang W-L, Lu Z, Bast Jr RC. The role of biomarkers in the management of epithelial ovarian cancer. Expert review of molecular diagnostics. 2017;17(6):577-91.

12. Alcazar JL, Castillo G, Jurado M, Garcia GL. Is expectant management of sonographically benign adnexal cysts an option in selected asymptomatic premenopausal women? Hum Reprod. 2005;20(11):3231-4.

13. Pascual MA, Graupera B, Hereter L, Rotili A, Rodriguez I, Alcazar JL. Intra-and interobserver variability of 2D and 3D transvaginal sonography in the diagnosis of benign versus malignant adnexal masses. Journal of Clinical Ultrasound. 2011;39(6):316-21.

14. Nunes N, Ambler G, Foo X, Widschwendter M, Jurkovic D. Prospective evaluation of IOTA logistic regression models LR1 and LR2 in comparison with subjective pattern recognition for diagnosis of ovarian cancer in an outpatient setting. Ultrasound in Obstetrics & Gynecology. 2018;51(6):829-35.

15. Hellstrom I, Raycraft J, Hayden-Ledbetter M, Ledbetter JA, Schummer M, McIntosh M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res. 2003;63(13):3695-700.

16. Moore RG, Brown AK, Miller MC, Skates S, Allard WJ, Verch T, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecologic oncology. 2008;108(2):402-8.

17. Chen X, Zhou H, Chen R, He J, Wang Y, Huang L, et al. Development of a multimarker assay for differential diagnosis of benign and malignant pelvic masses. Clin Chim Acta. 2015;440:57-63.

18. Moore RG, Miller MC, Disilvestro P, Landrum LM, Gajewski W, Ball JJ, et al. Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. Obstetrics and gynecology. 2011;118(2 Pt 1):280.

19. Moore RG, McMeekin DS, Brown AK, DiSilvestro P, Miller MC, Allard WJ, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecologic oncology. 2009;112(1):40-6.

20. Van Gorp T, Cadron I, Despierre E, Daemen A, Leunen K, Amant F, et al. HE4 and CA125 as a diagnostic test in ovarian cancer: prospective validation of the Risk of Ovarian Malignancy Algorithm. Br J Cancer. 2011;104(5):863-70.

21. Gentry-Maharaj A, Burnell M, Dilley J, Ryan A, Karpinskyj C, Gunu R, et al. Serum HE4 and diagnosis of ovarian cancer in postmenopausal women with adnexal masses. American journal of obstetrics and gynecology. 2020;222(1):56. e1-. e17.

22. Dochez V, Caillon H, Vaucel E, Dimet J, Winer N, Ducarme G. Biomarkers and algorithms for diagnosis of ovarian cancer: CA125, HE4, RMI and ROMA, a review. J Ovarian Res. 2019;12(1):28.

23. Dodge JE, Covens AL, Lacchetti C, Elit LM, Le T, Devries-Aboud M, et al. Preoperative identification of a suspicious adnexal mass: a systematic review and meta-analysis. Gynecologic oncology. 2012;126(1):157-66.

24. Partheen K, Kristjansdottir B, Sundfeldt K. Evaluation of ovarian cancer biomarkers HE4 and CA-125 in women presenting with a suspicious cystic ovarian mass. Journal of gynecologic oncology. 2011;22(4):244-52.

25. Gadducci A, Baicchi U, Marrai R, Ferdeghini M, Bianchi R, Facchini V. Preoperative evaluation of D-dimer and CA 125 levels in differentiating benign from malignant ovarian masses. Gynecol Oncol. 1996;60(2):197-202.

26. Jacobs I, Oram D, Fairbanks J, Turner J, Frost C, Grudzinskas J. A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. BJOG: An International Journal of Obstetrics & Gynaecology. 1990;97(10):922-9.

27. Seebacher V, Aust S, D'Andrea D, Grimm C, Reiser E, Tiringer D, et al. Development of a tool for prediction of ovarian cancer in patients with adnexal masses: Value of plasma fibrinogen. PLoS One. 2017;12(8):e0182383.

28. Yurkovetsky ZR, Linkov FY, D EM, Lokshin AE. Multiple biomarker panels for early detection of ovarian cancer. Future Oncol. 2006;2(6):733-41.

29. Timmerman D, Testa AC, Bourne T, Ferrazzi E, Ameye L, Konstantinovic ML, et al. Logistic regression model to distinguish between the benign and malignant adnexal mass before surgery: a multicenter study by the International Ovarian Tumor Analysis Group. Journal of Clinical Oncology. 2005;23(34):8794-801.

30. Simmons AR, Clarke CH, Badgwell DB, Lu Z, Sokoll LJ, Lu KH, et al. Validation of a Biomarker Panel and Longitudinal Biomarker Performance for Early Detection of Ovarian Cancer. Int J Gynecol Cancer. 2016;26(6):1070-7.

31. Clauss A. [Rapid physiological coagulation method in determination of fibrinogen]. Acta Haematol. 1957;17(4):237-46.

32. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988:837-45.

33. Lutz H. Model Assessment with ROC Curves. In: John W, editor. Encyclopedia of Data Warehousing and Mining, Second Edition. Hershey, PA, USA: IGI Global; 2009. p. 1316-23.

34. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology. 1983;148(3):839-43.

35. Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. pROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics. 2011;12:77.

36. Nolen BM, Lokshin AE. Biomarker testing for ovarian cancer: clinical utility of multiplex assays. Molecular diagnosis & therapy. 2013;17(3):139-46.

37. Lang S, Armstrong N, Deshpande S, Ramaekers B, Grimm S, de Kock S, et al. Clinically inappropriate post hoc exclusion of study participants from test accuracy calculations: the ROMA score, an example from a recent NICE diagnostic assessment. Ann Clin Biochem. 2019;56(1):72-81.

38. Group NG-O. The NCCP GP Ovarian Cancer Referral Guidelines HSE.ie2016 [Available from: https://www.hse.ie/eng/services/list/5/cancer/profinfo/resources/gpreferrals/gp%20ovarian%20cancer%20referral%20guidel

39. Moore RG, Jabre-Raughley M, Brown AK, Robison KM, Miller MC, Allard WJ, et al. Comparison of a novel multiple marker assay vs the Risk of Malignancy Index for the prediction of epithelial ovarian cancer in patients with a pelvic mass. American journal of obstetrics and gynecology. 2010;203(3):228. e1-. e6.

40. van den Akker PA, Aalders AL, Snijders MP, Kluivers KB, Samlal RA, Vollebergh JH, et al. Evaluation of the Risk of Malignancy Index in daily clinical management of adnexal masses. Gynecologic oncology. 2010;116(3):384-8.

41. Hilger WS, Magrina JF, Magtibay PM. Laparoscopic management of the adnexal mass. Clinical obstetrics and gynecology. 2006;49(3):535-48.

42. Bristow RE, Smith A, Zhang Z, Chan DW, Crutcher G, Fung ET, et al. Ovarian malignancy risk stratification of the adnexal mass using a multivariate index assay. Gynecologic oncology. 2013;128(2):252-9.

43. Moore LE, Pfeiffer RM, Zhang Z, Lu KH, Fung ET, Bast Jr RC. Proteomic biomarkers in combination with CA 125 for detection of epithelial ovarian cancer using prediagnostic serum samples from the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Cancer. 2012;118(1):91-100.

44. Moore RG, Blackman A, Miller MC, Robison K, DiSilvestro PA, Eklund EE, et al. Multiple biomarker algorithms to predict epithelial ovarian cancer in women with a pelvic mass: Can additional makers improve performance? Gynecol Oncol. 2019;154(1):150-5.

45. Cao Y, Ni X, Wang Y, Wang L, Yuan K, Gan G, et al. Clinical and prognostic significance of combined plasma fibrinogen concentrations and the monocyte-to-lymphocyte ratio in patients with ovarian cancer. Ann Transl Med. 2019;7(11):242.

46. Wu J, Fu Z, Liu G, Xu P, Xu J, Jia X. Clinical significance of plasma D-dimer in ovarian cancer: A meta-analysis. Medicine (Baltimore). 2017;96(25):e7062.

47. Polterauer S, Grimm C, Seebacher V, Concin N, Marth C, Tomovski C, et al. Plasma fibrinogen levels and prognosis in patients with ovarian cancer: a multicenter study. The oncologist. 2009;14(10):979-85.

48. Sakurai M, Satoh T, Matsumoto K, Michikami H, Nakamura Y, Nakao S, et al. High pretreatment plasma D-dimer levels are associated with poor prognosis in patients with ovarian cancer independently of venous thromboembolism and tumor extension. International Journal of Gynecologic Cancer. 2015;25(4):593-8.

49. Pang WW, Abdul-Rahman PS, Wan-Ibrahim WI, Hashim OH. Can the acute-phase reactant proteins be used as cancer biomarkers? Int J Biol Markers. 2010;25(1):1-11.

50. Ge L, Liu G, Hu K, Huang K, Zhang M, Zhou J, et al. A New Risk Index Combining d-Dimer, Fibrinogen, HE4, and CA199 Differentiates Suspecting Endometrial Cancer From Patients With Abnormal Vaginal Bleeding or Discharge. Technology in Cancer Research & Treatment. 2020;19:1533033819901117.

Tables

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Supplemental Tables

Table S1. Biomarker Levels in Benign and Borderline/Malignant cases for pre and postmenopausal women

Biomarker	**Benign**
	Premenopa
	n = 47
CA125 (IU/ml)	23.0 [11.5, 51
HE4 (pm/L)	47.3 [41.1, 60
D-Dimer (ng/ml)	446.3 [236.2,
Fibrinogen (g/L)	3.2 [2.8, 3.8]
ROMA (%)	7.6 [4.9, 11.8
RMI I	39.0 [12.5, 63
RMI II	52.0 [12.5, 10
	Postmenop
	n = 97
CA125 (IU/ml)	20.0 [12.0, 47
HE4 (pm/L)	65.7 [51.0, 83
D-Dimer (ng/ml)	489.3 [346.5,
Fibrinogen (g/L)	3.5 [3.0, 4.0]
ROMA (%)	17.0 [10.3, 28

Table S1. B

Table S1. Biomarker Levels in Benign and Borderline/Malignant cases for pre and postmenopausal women	**Table S1.** B
RMI I	108.0 [32.2, 2
RMI II	192.0 [44.0, 3
Variables are displayed as median [IQR], FDRp; False Discovery Rate P-value * Wilcoxon Rank Sum Test	Variables are

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Figure Titles

Figure 1

Corrected Partial AUCs of biomarkers for the prediction of borderline/malignant status in postmenopausal women

Figure S1

Boxplots of log of biomarker levels in pre- and post-menopausal women