RESEARCH | Article in Press

Towards a universal ovarian cancer biomarker model: clinical validation of Trx1 and CA125 dual strategy

Mi A Park · Songhak Kim · Xiaoguang Yang · Jong Am Song · Geon Woo Lee · Heon Jong Yoo · Soyoung Kim · Kyoung Hoon Suh · Young Bok Ko

Received: 13 August 2025 / Accepted: 26 August 2026
© The Author(s) 2026

Abstract

Background Ovarian cancer is the fifth leading cause of cancer-related mortality in women, with early detection being critical for improving patient outcomes. Current diagnostic biomarkers, including Cancer antigen 125 (CA125), human epididymis protein 4 (HE4), and their combined Risk of Ovarian Malignancy Algorithm (ROMA), exhibit limited sensitivity and specificity, particularly in early-stage disease and premenopausal women. Thioredoxin-1 (Trx1) has emerged as a potential complementary biomarker. This exploratory study evaluates the diagnostic utility of Trx1 in combination with CA125 and HE4.

Methods Serum levels of CA125, HE4, and Trx1 were measured in 80 ovarian cancer patients and 297 controls. The influence of age, menopausal status, obstetric history, pathological characteristics, and cancer stage on biomarker performance was analyzed. Diagnostic accuracy was assessed using receiver operating characteristic (ROC) curve analysis, with sensitivity, specificity, and area under the curve (AUC) values calculated for individual biomarkers and their combinations. Performance comparisons were conducted across premenopausal and postmenopausal subgroups.

Results Among individual biomarkers, CA125 demonstrated the highest diagnostic performance (AUC = 0.832), followed by HE4 (AUC = 0.812) and Trx1 (AUC = 0.768). The combination of Trx1 and CA125, termed the Dual-marker Ovarian Cancer Risk Algorithm (DORA), demonstrated diagnostic performance comparable to ROMA (AUC, 0.864 vs. 0.853) while showing higher sensitivity (87.5% vs. 57.5%). Trx1 concentrations showed minimal associations with age and menopausal status. In subgroup analyses, DORA demonstrated higher sensitivity than ROMA in premenopausal women (76.2% vs. 42.9%) and in patients with early-stage ovarian cancer (82.2% vs. 36.8–46.2%), while maintaining acceptable overall diagnostic performance.

Conclusion DORA demonstrated diagnostic performance comparable to that of ROMA while showing higher sensitivity in clinically relevant subgroups, including premenopausal women and patients with early-stage ovarian cancer. These findings suggest that incorporation of Trx1 may provide complementary diagnostic information in women undergoing evaluation for suspected ovarian malignancy.

Keywords Ovarian cancer · Early-stage ovarian cancer · Liquid biopsy · Thioredoxin 1 · Cancer antigen 125

This Article in Press is shared early to give you faster access to new research. It is citable and carries a permanent DOI. The final edited version will replace it automatically.

Mi A Park and Songhak Kim contributed equally to this work.

Published online: 03 September 2026

Abbreviations

WHO	World Health Organization
OC	Ovarian Cancer
CA125	Cancer Antigen 125
HE4	Human Epididymis Protein 4
Trx1	Thioredoxin 1
RMI	Risk of Malignancy Index
ROMA	Risk of Ovarian Malignancy Algorithm
ROC	Receiver Operating Characteristic
AUC	Area Under the Curve
EMR	Electronic Medical Records
SST	Serum Separating Tube
TNM	Tumor, Node, Metastasis

Background

Ovarian cancer is one of the most prevalent gynecological malignancies and ranks as the fifth leading cause of cancer-related mortality among women worldwide [1]. Unlike cervical cancer, whose incidence has declined due to vaccination programs, ovarian cancer remains a significant concern, with an increasing risk and a decreasing age of onset in some populations [1]. Due to the lack of early symptoms and effective screening methods, most ovarian cancer cases are diagnosed at an advanced stage, leading to poor prognoses. While early-stage ovarian cancer (Stages I and II) has a five-year survival rate of approximately 90%, this rate drops significantly to 20–40% in late-stage cases (Stages III and IV) [2, 3]. The pathogenesis of ovarian cancer remains incompletely understood, though genetic and endocrine factors have been identified as key contributors [4]. Therefore, improving diagnostic strategies that enable the timely identification of ovarian cancer, particularly in patients presenting with suspected ovarian pathology, is crucial for improving patient outcomes.

Among the various diagnostic approaches, blood-based biomarkers are considered the most practical and minimally invasive method for ovarian cancer detection. Biomarkers can facilitate early diagnosis, monitor disease progression, and assess treatment response. CA125 was the first biomarker widely used for ovarian cancer detection and monitoring following the discovery of the OC125 antibody in 1981 [5]. Although CA125 demonstrates substantial clinical utility, it has notable limitations. It is elevated in approximately 80% of ovarian cancer cases, however, its sensitivity for early-stage detection (Stages I and II) is limited to 50–60% [6, 7]. Furthermore, CA125 levels can be elevated in benign gynecological conditions (5%) and non-gynecological malignancies (28%), reducing its specificity and leading to potential false-positive results [7–9]. To improve diagnostic accuracy, several scoring systems, such as the Risk of Malignancy Index (RMI), which integrates CA125 levels with transvaginal ultrasound findings, have been developed [10].

To enhance diagnostic accuracy, HE4 was introduced as an additional ovarian cancer biomarker [11, 12]. First identified by Hellström et al. in 2003 [13], HE4 was approved by the FDA in 2009 for monitoring ovarian cancer progression [14]. Studies have demonstrated that combining HE4 with CA125 improves diagnostic performance compared to either biomarker alone [15]. The Risk of Ovarian Malignancy Algorithm (ROMA), which integrates HE4 and CA125 levels with menopausal status, was introduced to assess malignancy risk in women with pelvic masses [14]. FDA-approved in 2011, ROMA demonstrates high effectiveness in postmenopausal women but is less reliable in premenopausal populations [16]. Despite its clinical utility, HE4 levels can be affected by age, pregnancy, and non-gynecological conditions such as renal failure and lung cancer, increasing the false-positive rate in ROMA assessments [17–19]. Furthermore, recent studies suggest that neither HE4 alone nor ROMA consistently improves the detection of early-stage ovarian cancer compared with CA125 alone [20]. This underscores

the need for additional biomarkers that improve diagnostic performance, particularly in differentiating malignant from benign pelvic masses in women undergoing evaluation for suspected ovarian pathology.

Recently, Thioredoxin-1 (Trx1) has emerged as a promising biomarker for cancer detection, particularly in female malignancies. Trx1 is a redox-regulating protein involved in oxidative stress response and cellular signaling [21]. Recent studies have indicated that serum Trx1 levels are elevated in ovarian cancer patients who are CA125-negative, suggesting its potential as a complementary biomarker [22, 23]. Although Trx1 levels are also elevated in other malignancies, including breast [24, 25], gastric [26], and colorectal cancers [27], its strong association with ovarian cancer highlights its diagnostic potential. However, to be clinically effective, an ideal biomarker should demonstrate high sensitivity and specificity, regardless of patient demographics such as age or menopausal status. Previous studies have suggested that Trx1 expression may be less influenced by age or menopausal status [24].

Given these findings, further investigation of the diagnostic potential of Trx1 in ovarian cancer is warranted, both as a standalone biomarker and in combination with established biomarkers such as CA125. Therefore, this study aimed to evaluate the clinical performance of serum Trx1 and to assess whether a Trx1-CA125–based algorithm could serve as a complementary diagnostic tool for differentiating ovarian cancer from benign gynecologic conditions.

Methods

Participant selection and serum collection

Informed consent for the use of serum samples in research was obtained from all participants. Clinical samples were collected from patients visiting Chungnam National University (CNU) Hospital (Daejeon, South Korea), in accordance with the guidelines of the Independent Ethics Committee of the CNU College of Medicine (IRB approval no. 2022-04-048; 2024-12-029). Preoperative serum samples were collected from 80 ovarian cancer patients and 297 patients with benign gynecologic and adnexal conditions. The serum samples were provided by the biobank of CNU Hospital, a member of the Korean Biobank Network, and were retrospectively registered in the present study. Clinical data were retrospectively obtained from the Electronic Medical Records (EMR) system at CNU Hospital. To qualify for inclusion, participants had to meet the following criteria: (1) a confirmed pathological diagnosis of ovarian cancer; (2) no prior chemotherapy or radiotherapy before blood collection; (3) an assessment for hemolysis prior to serum collection to ensure sample quality; and (4) exclusion of patients with other existing malignant tumors. Blood samples were collected into Serum Separator Tubes (SST), allowed to clot, and then centrifuged at 1500 × g for 15 min. The resulting serum supernatant was stored at −80 °C in the CNU Hospital biobank. For this analysis, serum samples were completely thawed prior to testing. As this study was designed as an exploratory retrospective biomarker study using archived biobank specimens, a formal prospective sample size calculation was not performed. All available archived serum samples that met the predefined inclusion and exclusion criteria were included in this exploratory analysis. To assess the adequacy of the study population, a post-hoc power analysis was performed using MedCalc software (v23.6.1, Ostend, Belgium). Based on the observed difference in serum Trx1 concentrations between ovarian cancer patients and controls, the available sample size provided a statistical power of >99% at a two-sided significance level of 0.05.

Quantification of biomarker levels in serum

Three biomarkers were analyzed in this study: CA125, HE4, and Trx1. Among these, Trx1 was the only biomarker detected using a non-commercial kit, requiring a more specific detection process. CA125 and HE4 levels were measured using commercially available kits, with CA125 tested manually and HE4 using a fully automated detector.

Serum Trx1 concentrations were measured using a manufacturer-developed sandwich ELISA for Trx1 quantification (E&S Healthcare, Daejeon, Republic of Korea), based on the previously described Trx1 immunoassay platform [25]. The assay employs monoclonal antibodies generated using hybridoma technology. The antibody pair was selected based on binding affinity and analytical performance during assay development. The analytical procedure was adapted for the present study and performed according to the manufacturer's protocol. The assay underwent analytical validation prior to clinical application in accordance with predefined acceptance criteria. The assay demonstrated acceptable analytical performance, with intra-assay and inter-assay coefficients of variation of ≤ 10% and ≤ 20%, respectively. Briefly, serum samples or purified Trx1 protein (used as a calibrator, positive control, and negative control) were pre-mixed with detection antibodies (anti-Trx1 monoclonal antibody conjugated with peroxidase; clone B264) and adjusted to 100 µL using PBSA. In parallel, 100 µL of the prepared samples was added to wells coated with capture antibody (clone B266-1) on a 96-well plate. The reaction was allowed to proceed for one hour, resulting in the formation of antigen-antibody complexes. After three washes with a washing solution, TMB (3,3′,5,5′-tetramethylbenzidine) was used as the substrate. The enzymatic reaction was halted with the addition of 2 N sulfuric acid, causing a color change from blue to yellow. The intensity of the color change was measured at 450 nm using a microplate reader. A standard curve was constructed based on absorbance values of various known concentrations of recombinant human Trx1 protein, which was used to determine the Trx1 concentration in the serum samples.

CA125 levels in serum were measured using a sandwich ELISA with the Accubind kit (Monobind Inc., California, USA), following the manufacturer's protocol. Quality control parameters, as outlined in the manufacturer's protocol, ensured test validity. Each sample was tested in duplicate, and the mean absorbance value was used for analysis.

HE4 levels in serum were measured using the Elecsys HE4 assay (Roche, Germany) on a Cobas 8000 e602 analyzer (Roche, Germany), in accordance with the manufacturer's instructions. All serum samples were analyzed in a blinded manner, and laboratory personnel were unaware of the clinical diagnosis at the time of biomarker measurement.

Algorithm and calculation of biomarker combinations

Four combinations of the three biomarkers were investigated in this study. The specific biomarker combinations and the associated calculation formulas used to assess diagnostic performance are detailed in Table 4S. Two combinations warrant particular mention: the ROMA index (CA125 + HE4) and the CA125 + Trx1 combination (Dual-marker Ovarian Cancer Risk Algorithm, DORA) index.

The ROMA index was calculated using the following formulas, as referenced from its commercial diagnostic kit:

- Premenopausal ROMA index formula: $12 + (2.38 \times \ln(HE4)) + (0.062 \times \ln(CA125))$
- Postmenopausal ROMA index formula: $8.09 + (1.04 \times \ln(HE4)) + (0.732 \times \ln(CA125))$

In this study, multivariate logistic regression analysis was applied to evaluate the association between CA125 and Trx1 concentrations and the likelihood of ovarian cancer. This method was selected to estimate the individual contributions of these biomarkers to the binary outcome. Ovarian cancer cases were designated as the positive class and encoded as 1, whereas benign control samples were encoded as 0. The independent variables included log-transformed serum levels of CA125 and Trx1. Internal validation was performed using a repeated random train-test splitting strategy. Specifically, the dataset was randomly partitioned into training and testing sets at a 7:3 ratio and repeated 30 times using different random seeds (0–29). Model performance was summarized by calculating the mean and standard deviation of accuracy, sensitivity, and specificity across all iterations. Logistic regression models were constructed using the training datasets, and predicted probabilities (PPs) for ovarian cancer were subsequently calculated for the corresponding test datasets. PPs ranged from 0 to 100% for each model. Binary classification was performed using the default probability threshold of 0.5 implemented in the scikit-learn

LogisticRegression classifier. Samples with PP values below 0.5 were classified as negative, whereas samples with PP values ≥ 0.5 were classified as positive. Sensitivity and specificity were subsequently calculated for each iteration. For the final diagnostic evaluation, however, sensitivity and specificity reported in the manuscript were calculated using the optimal DORA score cutoff (46.86), which was determined by ROC curve analysis to achieve the best balance between sensitivity and specificity.

ROC analysis

Receiver Operating Characteristic (ROC) analyses were conducted using MedCalc software to assess the diagnostic accuracy of each biomarker signature. Univariate ROC analysis was performed for each biomarker combination to determine the ROC curve, area under the curve (AUC), standard error (SE) of the AUC, and 95% confidence interval (CI) [28]. Statistical significance was determined by testing whether the AUC differed significantly from 0.5, with $p < 0.05$ considered statistically significant.

Decision curve analysis and clinical impact curve analysis

DCA was conducted according to the method described to evaluate the net clinical benefit of each algorithm across a range of threshold probabilities [29]. Net benefit was calculated as:

$$Net\ Benefit = \frac{TP}{N} - \frac{FP}{N} \times \frac{Pt}{1 - Pt}$$

where TP represents the number of true-positive cases, FP represents the number of false-positive cases, N is the total number of subjects, and Pt is the selected threshold probability. Given the exploratory nature of this proof-of-concept study, analyses focused primarily on threshold probabilities ranging from 5% to 15%, which are considered clinically relevant for high-sensitivity diagnostic and triage settings.

CIC analysis was performed to estimate the number of individuals classified as high risk and the number of true ovarian cancer cases identified per 1,000 tested subjects across different threshold probabilities. Exploratory subgroup analyses were additionally performed in patients with FIGO stage I–II ovarian cancer.

All statistical analyses were performed using Python (version 3.11; Python Software Foundation, DE, USA). DCA and CIC analyses were implemented using custom scripts based on the methodology of Vickers et al., and figures were generated using the Matplotlib library [30].

Data analysis

Immunoassay experiments were independently performed in duplicate. Continuous variables are presented as median and interquartile range (IQR), unless otherwise specified. Statistical analyses were performed using GraphPad Prism 10 (v10.2.0, GraphPad Software, CA, USA). Because biomarker concentrations were not normally distributed, comparisons between two groups were performed using the Mann–Whitney U test, whereas comparisons among three or more groups were conducted using the Kruskal–Wallis test. A two-sided p-value < 0.05 was considered statistically significant. In the figures, *, **, and *** indicate $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, whereas ns denotes non-significant differences.

Results

Distribution of demographic clinical variables in participants

This study analyzed the clinical characteristics of 80 ovarian cancer patients and 297 patients with benign gynecologic conditions, as summarized in Table S1. The collected demographic data included age, menopausal status, and obstetric history, while clinical information covered histopathological classification and FIGO staging.

The mean age of ovarian cancer patients was 58.7 ± 14.3 years, compared to 49.3 ± 12.1 years for the control group, indicating a statistically significant difference between the two groups. Among patients with ovarian cancer, cases were relatively evenly distributed across age groups from the fourth to seventh decades of life, whereas the largest proportion of control subjects was observed among women in their forties (27.61%), followed by those aged ≤ 30 years (26.26%) and in their fifties (24.58%).

Menopausal status analysis revealed that the majority of ovarian cancer patients (73.75%) were postmenopausal, while the control group exhibited a higher proportion of premenopausal individuals (69.36%). Obstetric history assessment indicated that a larger proportion of ovarian cancer patients (86.25%) had a history of childbirth compared to the control group (69.36%), suggesting a potential correlation between reproductive factors and ovarian cancer risk.

Histopathological classification identified serous carcinoma as the predominant subtype, accounting for 52.50% of ovarian cancer cases, followed by non-serous types in 41.25% of cases. The classification for 6.25% of patients remained undetermined. The observed distribution aligns with the latest WHO classification, where high-grade serous carcinoma constitutes the most prevalent subtype (70%), followed by endometrioid (10%), clear cell (6–10%), low-grade serous (5%), and mucinous carcinoma (3–4%) [31, 32]. This suggests that the study cohort reflects a representative distribution of ovarian cancer subtypes. The benign control cohort consisted primarily of patients with benign ovarian disorders (74.07%), including serous cystadenoma, mature cystic teratoma, and parovarian cysts, while the remaining patients presented with benign uterine or cervical conditions (25.93%), such as endometrial polyps, endometriosis, and uterine prolapse. Therefore, the control cohort was designed to reflect benign gynecologic and adnexal lesions commonly encountered in routine clinical practice. FIGO staging analysis indicated that 43.75% of ovarian cancer patients were diagnosed at early stages (Stages I and II), whereas 42.50% had advanced-stage disease (Stages III and IV). Staging information was unavailable for 11 patients.

Comparative clinical performance of individual biomarkers

To assess the diagnostic utility of CA125, HE4, and Trx1 for ovarian cancer, their serum levels were analyzed in 377 participants. The ovarian cancer group ($n = 80$) exhibited significantly elevated median levels of CA125 (60.38 U/mL), HE4 (110.50 pmol/L), and Trx1 (28.03 U/mL) compared to the control group ($n = 297$), where the respective values were 1.80 U/mL, 47.50 pmol/L, and 23.48 U/mL (Table S2). All biomarkers demonstrated statistically significant differences between cases and controls ($P < 0.001$ for CA125 and HE4, $P < 0.05$ for Trx1) (Fig. 1A). ROC curve analysis showed AUC values of 0.832 (CA125), 0.812 (HE4), and 0.768 (Trx1) (Fig. 1B), confirming that CA125 had the highest diagnostic performance, followed by HE4 and Trx1 (Fig. 1C). Based on optimal cut-off values, CA125 had a sensitivity of 53.75% and specificity of 93.60%, while HE4 showed 73.75% sensitivity and 80.13% specificity. Trx1 demonstrated 62.50% sensitivity and 80.81% specificity (Table S3). These findings support further evaluation of biomarker combinations to improve the diagnostic performance for ovarian cancer.

Biomarker levels were further analyzed based on age, menopausal status, and childbirth history. HE4 showed a significant positive correlation with age in both ovarian cancer ($r = 0.367$, $P < 0.001$) and control groups ($r = 0.396$, $P < 0.001$), while CA125 exhibited a weaker but significant positive correlation in ovarian cancer patients ($r = 0.258$, $P < 0.05$) and a negative correlation in controls ($r = -0.194$, $P < 0.01$). Trx1 levels showed minimal association with age in both ovarian cancer and control groups (Fig. S1A, Table S5). Menopausal status

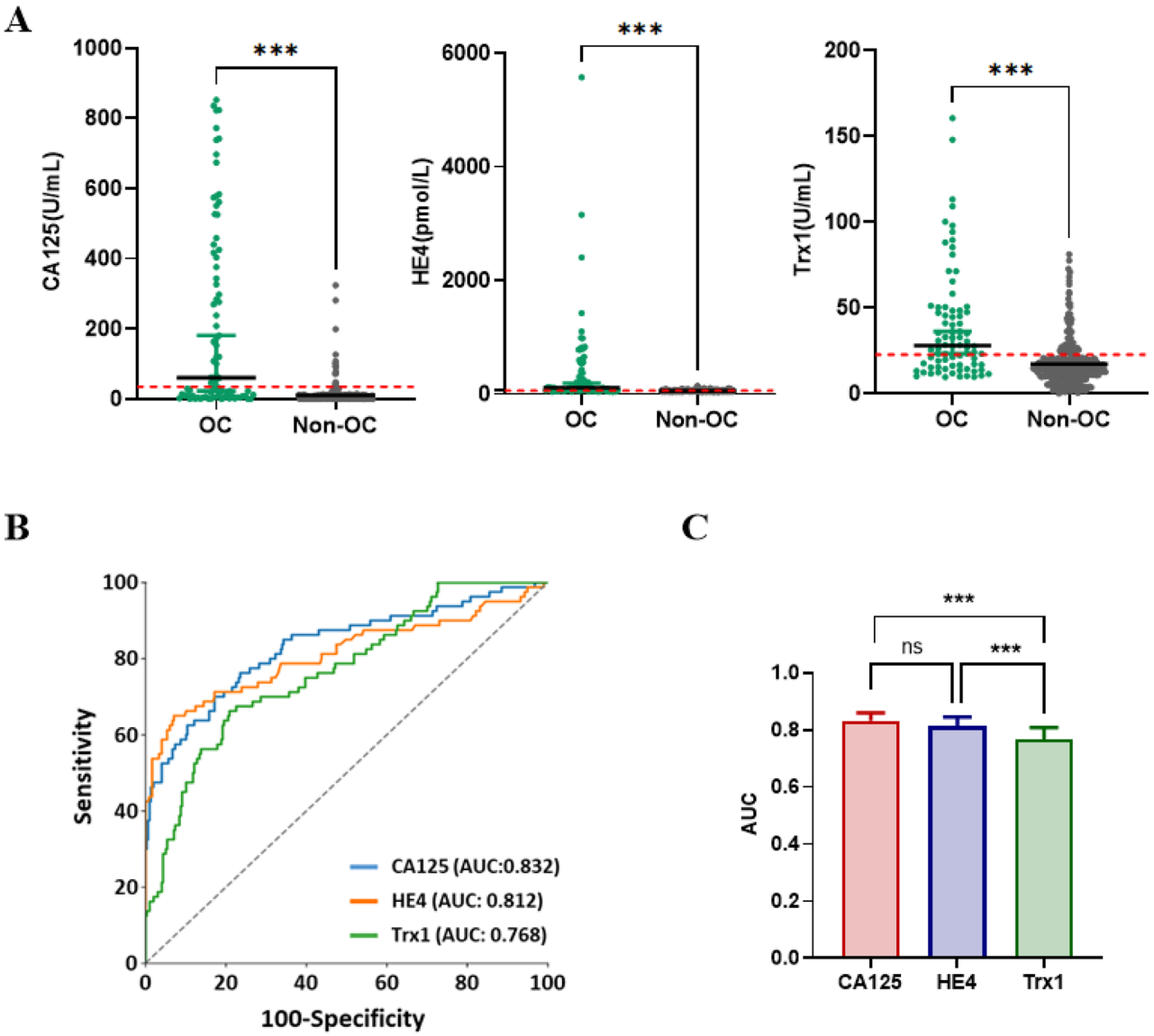


Fig. 1 Clinical performance analysis for individual biomarkers for ovarian cancer. The clinical performance of CA125, HE4, and Trx1 was evaluated in 80 ovarian cancer patients and 297 non-cancer patients. **A** Serum levels of CA125, HE4, and Trx1 in study participants. Black horizontal lines indicate the median values, while red dotted lines represent biomarker cut-off levels. **B** ROC curve analysis of CA125, HE4, and Trx1. **C** Comparison of AUC values for the three biomarkers. A single asterisk (*) indicates $p<0.05$, three asterisks (**) indicate $p<0.001$, and 'ns' denotes non-significant differences

significantly affected CA125 and HE4 levels, but not Trx1, particularly in ovarian cancer patients (Fig. S1B). Similarly, CA125 and HE4 levels varied significantly with childbirth history in controls, whereas Trx1 remained unaffected (Fig. S1C). These findings suggest that Trx1 levels are less influenced by demographic variables such as age, menopausal status, and childbirth history than CA125 and HE4, supporting its potential role as a complementary biomarker in ovarian cancer diagnosis.

Expression patterns of histopathological and clinical stage associations were further analyzed between serous and non-serous ovarian cancer cases ($n=80$). CA125 and HE4 exhibited significantly higher expression in serous tumors than in non-serous types ($P<0.001$). However, Trx1 showed no significant variation based on histopathological classification (Fig. 2). Importantly, in relation to FIGO staging, CA125 and HE4 levels were significantly elevated in advanced-stage ovarian cancer (Stages III–IV) compared to early-stage cases (Stages I–II). In

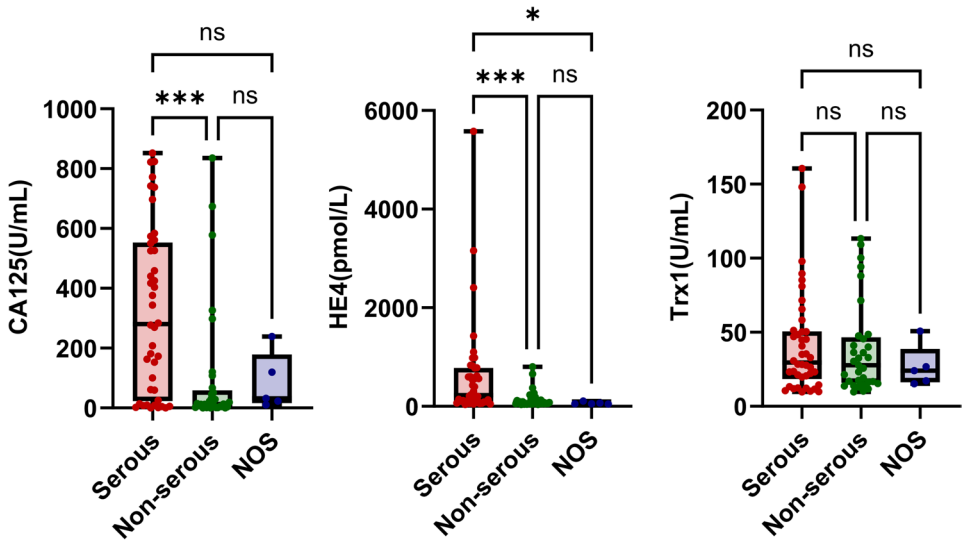


Fig. 2 Correlation analysis between pathological results and biomarker levels in ovarian cancer patients. This analysis examines biomarker expression according to the pathological subtypes of ovarian cancer. The "Serous" category includes high-grade serous ovarian carcinoma, low-grade serous ovarian carcinoma, and borderline serous ovarian carcinoma. Box-and-whisker plots show the median (center line), interquartile range (box), and min to max range (whiskers). Individual dots represent individual patients. The "Non-serous" category encompasses mucinous, clear cell, granulosa cell, and endometrioid carcinomas. "NOS" stands for "Not Otherwise Specified." A single asterisk (*) indicates a *p*-value of less than 0.05, two asterisks (**) indicate a p-value of less than 0.01, and three asterisks (***) indicate a *p*-value of less than 0.001. "ns" denotes a statistically non-significant result

contrast, Trx1 expression did not differ significantly between early and advanced stages (Fig. 3). This suggests that Trx1 may complement CA125 and HE4 by being less influenced by tumor type and stage, potentially providing complementary diagnostic information across different histological subtypes and disease stages.

Comparison of clinical performance of biomarker combinations

The diagnostic performance of four biomarker combinations was evaluated using logistic regression models (Table S4). The evaluation included ROC curve analysis, AUC, clinical sensitivity, and specificity. The performance of these combinations was compared with the existing ROMA algorithm (CA125 + HE4), with a focus on incorporating Trx1 into biomarker combinations for diagnostic evaluation while considering patient age, menopausal status, and overall clinical applicability.

The ROC curve analysis (Fig. 4A) revealed that the Trx1 + CA125 + HE4 combination achieved the highest overall diagnostic performance, with a sensitivity of 87.50% and a specificity of 95.50%. The Trx1 + CA125 combination demonstrated a sensitivity of 87.50% and a specificity of 90.90%, compared with 57.50% sensitivity and 93.27% specificity for the ROMA algorithm. In contrast, the Trx1 + HE4 combination exhibited the lowest diagnostic performance among the evaluated models. Statistical analysis confirmed that the AUC for Trx1 + HE4 was significantly lower than for Trx1 + CA125 + HE4 and CA125 + HE4 (Fig. 4B). Given its comparable overall diagnostic performance and simpler biomarker composition, the Trx1 + CA125 combination was selected for further evaluation.

Comparative analyses according to age, menopausal status, and disease stage demonstrated that ROMA showed significant associations with age, whereas DORA exhibited minimal associations with age (Fig. S2A). In addition, ROMA performance varied according to menopausal status, whereas DORA showed less variation between premenopausal and postmenopausal groups (Fig. S2B). These findings suggest that DORA may provide complementary diagnostic information that is less influenced by demographic characteristics. Among

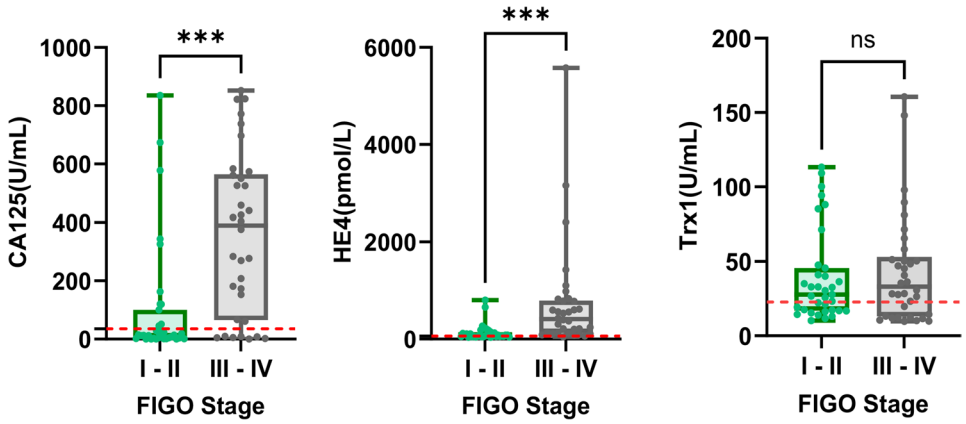


Fig. 3 Analysis of biomarker expression patterns according to ovarian cancer FIGO stage progression. Box-and-whisker plots displaying the expression levels of CA125, HE4, and Trx1 in early-stage (I-II) and late-stage (III-IV) ovarian cancer. Box-and-whisker plots show the median (center line), interquartile range (box), and min to max range (whiskers). Individual dots represent individual patients. Three asterisks (***) indicate a *p*-value of less than 0.001. "ns" denotes a statistically non-significant result

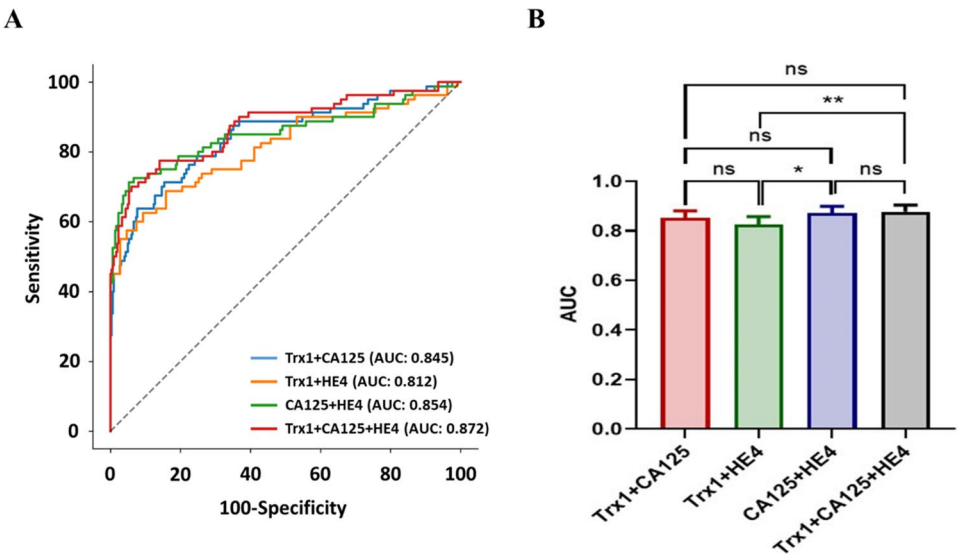


Fig. 4 Clinical performance analysis of combined biomarkers for ovarian cancer. **A** AUC analysis from logistic regression using Trx1, CA125, and HE4 for multi-marker validation. **B** Comparison of AUC values for combined biomarkers. A single asterisk (*) indicates a *p*-value of less than 0.05, two asterisks (**) indicate a *p*-value of less than 0.01, and "ns" denotes a statistically non-significant result

premenopausal women, ROMA demonstrated a sensitivity of 42.86%, a specificity of 91.43%, and an AUC of 0.737. In postmenopausal women, ROMA achieved a sensitivity of 62.71%, a specificity of 97.75%, and an AUC of 0.869. In comparison, DORA demonstrated sensitivities of 76.19% and 91.53% in premenopausal and postmenopausal women, respectively, with corresponding AUC values of 0.734 and 0.880. DORA also showed higher sensitivities in both early-stage (82.22%) and late-stage (94.29%) ovarian cancer than ROMA (Table 1).

Overall, DORA demonstrated diagnostic performance comparable to that of ROMA while showing higher sensitivity in clinically relevant subgroups, including premenopausal women and patients with early-stage ovarian cancer. These findings suggest that DORA may serve as a complementary diagnostic tool for differentiating ovarian cancer from benign gynecologic conditions.

Table 1 Comparison of clinical performance between ROMA and DORA

Variables	Conditions	Cut off	Sensitivity (95% CI)	Specificity (95% CI)	AUC (95% CI)	*P*-value
ROMA	*Premenopausal status*	11.40	42.86 (21.8–66.0)	91.43 (86.86–94.51)	0.737 (0.612–0.863)	0.0002
	Early stage (I-II)		36.84 (19.15–58.96)	-	0.710 (0.575–0.845)	<0.0023
	Late stage (III-IV)		42.86 (9.9–81.6)	-	0.839 (0.782–0.887)	0.0001
	Postmenopausal status	29.90	62.71 (49.95–73.92)	97.75 (92.17–99.38)	0.869 (0.808–0.930)	<0.0001
	Early stage (I-II)		46.15 (28.75–64.54)	-	0.825 (0.723–0.928)	0.0001
	Late stage (III-IV)		75.00 (57.89–86.75)		0.928 (0.866–0.990)	<0.0001
	All	-	57.50 (46.57–67.74)	93.27 (89.83–95.60)	0.853 (0.774–0.921)	<0.0001
DORA	*Premenopausal status*	46.86	76.19 (54.91–89.37)	88.63 (83.63–92.24)	0.734 (0.608–0.860)	0.0003
	Postmenopausal status		91.53 (81.65–96.32)	96.55 (90.34–98.82)	0.880 (0.821–0.939)	<0.0001
	Early stage (I-II)		82.22 (68.67–90.71)	-	0.780 (0.696–0.864)	<0.0001
	Late stage (III-IV)		94.29 (81.39–98.42)	-	0.921 (0.858–0.984)	<0.0001
	All		87.50 (78.50–93.07)	90.90 (87.10–93.68)	0.864 (0.805–0.916)	<0.0001

Decision curve analysis and clinical impact curve analyses of DORA in early-stage ovarian cancer

To further explore the potential clinical utility of DORA, which incorporates circulating Trx1, decision curve analysis (DCA) and clinical impact curve (CIC) analyses were performed in patients with early-stage (FIGO stage I–II) epithelial ovarian cancer ($n=45$) and benign control subjects ($n=297$) (Fig. 6). The DCA demonstrated that DORA provided greater net clinical benefit than the conventional ROMA algorithm across clinically relevant threshold probabilities, particularly within the 5%–15% range. The greatest difference in net benefit was observed at approximately the 10% threshold probability, suggesting that incorporation of Trx1 may provide complementary diagnostic information in the evaluation of early-stage ovarian cancer.

The CIC analysis further illustrated the potential clinical impact of DORA by identifying a greater number of true-positive early-stage ovarian cancer cases than ROMA across clinically relevant threshold probabilities. At a threshold probability of 10%, DORA identified 37 of 45 patients with early-stage ovarian cancer, whereas ROMA identified 29 cases. This improvement in sensitivity was accompanied by an increased number of individuals classified as high risk, reflecting the expected trade-off between sensitivity and specificity. Taken together, these findings suggest that incorporation of circulating Trx1 into a CA125-based diagnostic algorithm may improve the identification of early-stage ovarian cancer in selected clinical settings and may provide additional clinical utility as an adjunctive diagnostic tool.

Discussion

The ROMA algorithm, which combines CA125 and HE4, has been widely used in ovarian cancer diagnostics, particularly among postmenopausal women. However, HE4, a product of the WFDC2 gene, is not specific to ovarian cancer and can be elevated in other conditions, such as endometrial cancer, lung tumors, and

inflammatory diseases, including COVID-19 and sepsis [33–41]. Additionally, HE4 levels fluctuate with age and menopausal status, leading to potential misclassification in diagnosis [16, 17]. To address some of these limitations, we developed the DORA algorithm, incorporating Trx1, a redox-regulating protein involved in cancer progression [21]. Trx1 has been investigated as a biomarker in various cancers, including breast, colon, and gastric cancers [42–49], and previous studies have suggested that its expression is less affected by age, TNM stage, and menopausal status [22, 24, 25]. Consequently, we developed the DORA algorithm, which combines Trx1 and CA125 to evaluate whether incorporation of Trx1 could provide complementary diagnostic information while maintaining overall diagnostic performance. The incorporation of Trx1 as a biomarker may offer several potential advantages. In the present cohort, Trx1 concentrations showed minimal associations with age, menopausal status, and childbirth history (Fig. S1). Furthermore, in exploratory subgroup analyses, incorporation of Trx1 into the DORA algorithm was associated with higher sensitivity in clinically relevant subgroups, particularly among patients with early-stage ovarian cancer and premenopausal women (Fig. S2).

In this exploratory retrospective study, the DORA algorithm demonstrated higher sensitivity compared with ROMA in clinically relevant subgroups, particularly among premenopausal women and patients with early-stage ovarian cancer, while maintaining overall diagnostic performance. Previous studies have suggested that clinically useful ovarian cancer risk algorithms should maintain sensitivity > 80% while preserving specificity ≥ 75% [50–53]. In our study, ROMA demonstrated a sensitivity of 42.9% and specificity of 91.4% in premenopausal women, and 62.7% and 97.8% in postmenopausal women. In contrast, DORA showed higher sensitivity, reaching 76.2% in premenopausal women and 91.5% in postmenopausal women, with specificity values of 88.6% and 96.6%, respectively (Fig. 5; Table 1). Although DORA had slightly reduced specificity compared to ROMA, its higher sensitivity may facilitate the detection of ovarian cancer, particularly in non-serous subtypes where CA125 and HE4 perform poorly (Fig. 2). These findings suggest that Trx1 may provide complementary diagnostic information, particularly for ovarian cancer subtypes that are more difficult to detect using conventional biomarkers.

In the present study, Trx1 expression showed no significant differences according to FIGO stage, whereas CA125 and HE4 levels were significantly elevated in advanced-stage disease (Fig. 3). This observation suggests that Trx1 may provide complementary diagnostic information across different stages of ovarian cancer. Notably, exploratory subgroup analyses demonstrated higher sensitivity of DORA among patients with early-stage ovarian cancer, although these findings should be interpreted cautiously because early-stage detection was not a predefined objective of model development.

HE4 concentrations are known to be influenced by age and several non-malignant conditions, including renal dysfunction and chronic inflammatory diseases [54–57]. Similarly, Trx1 expression has been associated with inflammatory and immune-related disorders, as well as tumor development and progression [43, 58–61]. In the present study, the control cohort consisted of patients with benign gynecologic and adnexal conditions rather than healthy individuals. As a result, elevated Trx1 concentrations observed in some benign conditions may have contributed to the relatively lower specificity of Trx1 compared with CA125 and HE4. Despite this limitation, DORA maintained overall diagnostic performance comparable to ROMA while demonstrating higher sensitivity in clinically relevant subgroups (Figs. 2 and 3). These findings suggest that incorporation of Trx1 may provide complementary diagnostic information in the evaluation of patients with suspected ovarian malignancy.

Although Trx1 alone exhibited limited diagnostic performance, incorporation of Trx1 into the DORA algorithm was associated with higher sensitivity in clinically relevant subgroups, particularly among premenopausal women and patients with early-stage ovarian cancer (Table 1 and Fig. 5). Early diagnosis of ovarian cancer has important implications for both patient outcomes and healthcare burden. Notably, ROMA is known to demonstrate reduced sensitivity in premenopausal women [16], representing a clinically important limitation because ovarian cancer in younger women may be more difficult to recognize. In the present study, DORA showed higher sensitivity in premenopausal women while maintaining acceptable overall diagnostic performance. In addition, unlike ROMA, which requires separate equations according to menopausal status, DORA utilizes a single equation irrespective of age or menopausal status (Table S4). This simplified approach may facilitate clinical implementation and reduce potential errors related to menopausal classification. Although incorporation of Trx1 into

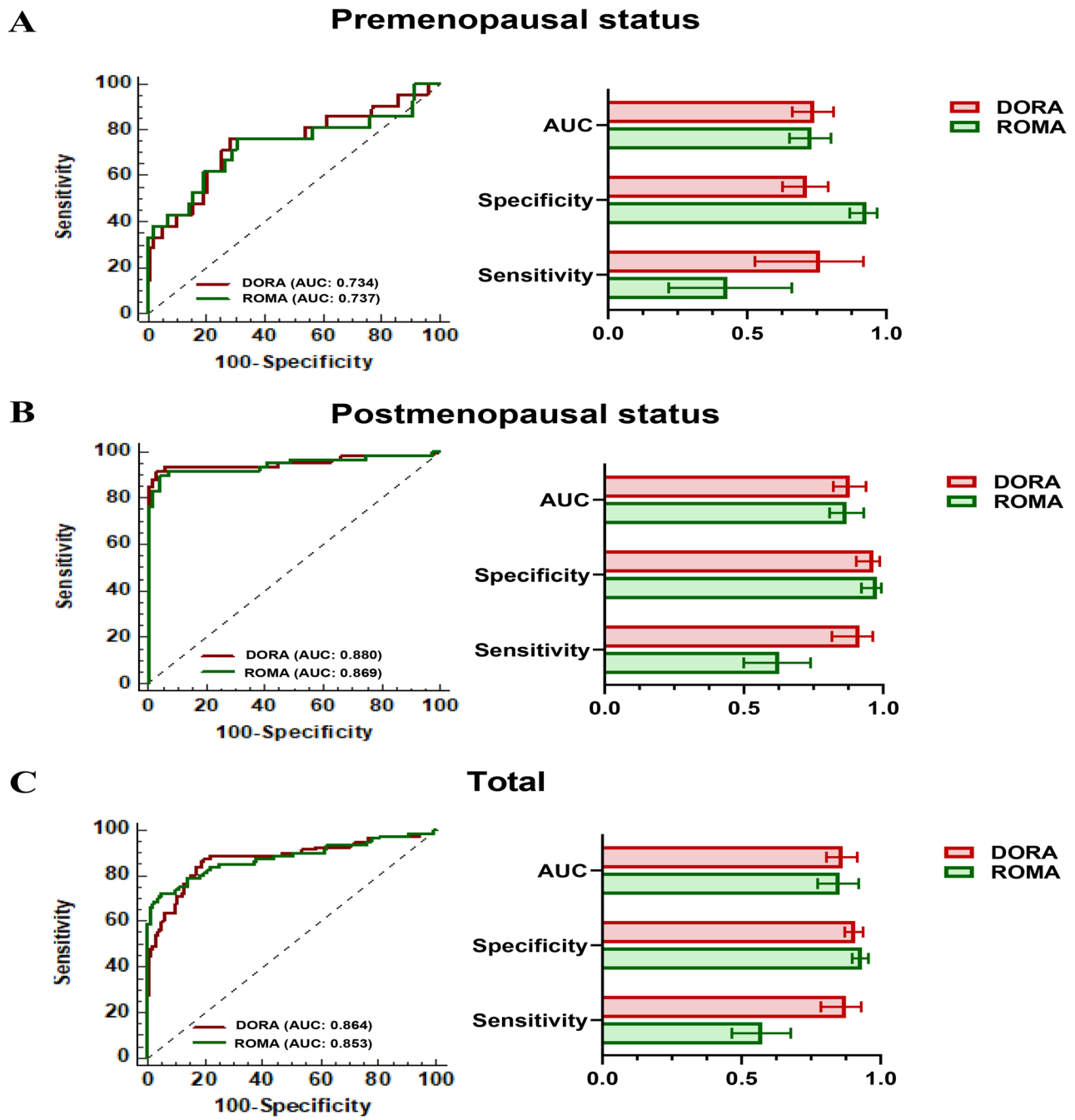


Fig. 5 Comparison of clinical performance between ROMA and DORA by menopausal status. **A** ROC analysis results for 231 premenopausal subjects. **B** ROC analysis results for 146 postmenopausal subjects. **C** ROC analysis results for the combination of ROMA and DORA in all 377 subjects. Bars represent sensitivity, specificity, and AUC values. Error bars indicate 95% confidence intervals

DORA did not substantially improve overall discriminatory performance, even modest improvements in sensitivity may provide clinically meaningful benefits by reducing missed cancer diagnoses. However, these gains were accompanied by reductions in specificity, and the clinical significance of this trade-off warrants further prospective evaluation.

In exploratory analyses, decision curve analysis (DCA) and clinical impact curve (CIC) analyses provided additional information regarding the potential clinical utility of DORA (Fig. 6). DCA demonstrated greater net

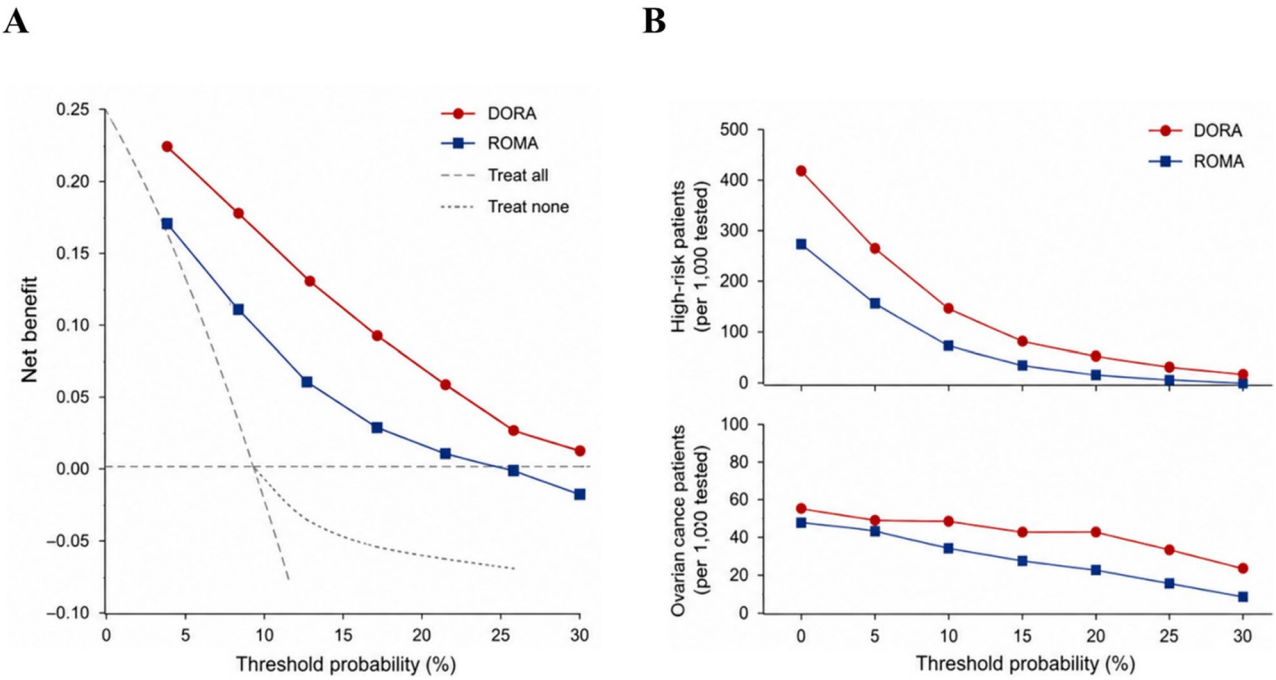


Fig. 6 Decision curve analysis and clinical impact curve comparing DORA and ROMA in patients with early-stage (FIGO I–II) ovarian cancer. **A** Decision curve analysis (DCA) comparing the net clinical benefit of DORA and ROMA across a range of threshold probabilities. The gray dashed line represents the strategy of treating all patients as having ovarian cancer, whereas the gray dotted line represents the strategy of treating no patients. A higher net benefit indicates greater potential clinical utility. **B** Clinical impact curve (CIC) illustrating the estimated clinical consequences of applying DORA and ROMA across the same threshold probabilities. The upper panel shows the estimated number of patients classified as high risk per 1,000 individuals evaluated, and the lower panel shows the estimated number of true-positive ovarian cancer cases among those classified as high risk

clinical benefit than ROMA across lower threshold probabilities in patients with early-stage ovarian cancer, while CIC suggested that DORA could identify a greater number of true-positive early-stage ovarian cancer cases than ROMA, albeit with an accompanying increase in false-positive classifications. These findings should be considered hypothesis-generating rather than confirmatory. Nevertheless, they suggest that incorporation of Trx1 may provide complementary diagnostic information in clinical settings where maximizing diagnostic sensitivity is considered particularly important.

Several limitations of this study should be acknowledged. First, this was a retrospective case-control study conducted at a single institution using an enriched disease cohort. Therefore, the present study was not designed to evaluate DORA as a screening tool in asymptomatic, low-prevalence populations. Rather, DORA was developed as an adjunctive diagnostic tool intended to assist clinicians in differentiating ovarian cancer from benign gynecologic and adnexal lesions in women undergoing clinical evaluation for suspected ovarian pathology. Consequently, the diagnostic performance observed in the present study may not fully reflect that achieved in real-world clinical settings with lower disease prevalence. Second, although DORA demonstrated promising diagnostic performance, particularly in clinically relevant subgroups, the findings were derived from an exploratory retrospective cohort and should be interpreted cautiously. External validation in independent cohorts and prospective evaluation in clinically representative populations are required before routine clinical implementation can be recommended. Third, the study population consisted exclusively of Korean women recruited from a single center, which may limit the generalizability of the findings. Therefore, additional multicenter studies involving larger and ethnically diverse populations are warranted.

Conclusions

The DORA algorithm demonstrated diagnostic performance comparable to that of ROMA while showing higher sensitivity in clinically relevant subgroups. These findings suggest that incorporation of Trx1 may provide complementary diagnostic information in the evaluation of patients with suspected ovarian malignancy. Nevertheless, prospective studies and external validation are required to confirm these findings and to determine the clinical utility of DORA in routine practice.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1186/s12885-026-16887-2.

Acknowledgements We would like to express our sincere gratitude to the Innovation Center for Industrial Mathematics, National Institute for Mathematical Sciences for their invaluable support and contributions to this study. Additionally, we extend our appreciation to the Chungnam National University Hospital Biobank for providing essential resources and assistance with sample collection and management. Their support has been instrumental in the successful completion of this research.

Authors' contributions KHS and YBK conceptualized the study. YBK supervised the study. MAP curated the data. SHK analyzed the data. SHK and XY interpreted the results. SHK and MAP drafted the manuscript. All authors have reviewed and approved the submitted version of the manuscript.

Funding This study was supported by the 2024 Chungnam National University Hospital Research Fund (2024-CF-008). S.K. were supported by the National Institute for Mathematical Sciences (NIMS) grant funded by the Korean government (MSIT) (grant no. NIMS-B25810000).

Data availability Due to institutional and ethical restrictions associated with patient-derived clinical data, individual-level datasets are not publicly available but may be made available from the corresponding author upon reasonable request and with appropriate institutional approval.

Declarations

Ethics approval and consent to participate This study was approved by the Institutional Review Board of Chungnam National University Health System (IRB approval no. 2022-04-048; 2024-12-029). Written informed consent for the use of blood samples in research was obtained from all patients, and the study was conducted in accordance with the Declaration of Helsinki.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17–48.
2. Zeppernick F, Meinhold-Heerlein I. The new FIGO staging system for ovarian, fallopian tube, and primary peritoneal cancer. Arch Gynecol Obstet. 2014;290(5):839–42.

3. Schiavone MB, Herzog TJ, Lewin SN, Deutsch I, Sun X, Burke WM, et al. Natural history and outcome of mucinous carcinoma of the ovary. Am J Obstet Gynecol. 2011;205(5):e4801–8.
4. Holschneider CH, Berek JS. Ovarian cancer: epidemiology, biology, and prognostic factors. Semin Surg Oncol. 2000;19(1):3–10.
5. Bast RC, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981;68(5):1331–7.
6. Munkarah A, Chatterjee M, Tainsky MA. Update on ovarian cancer screening. Curr Opin Obstet Gynecol. 2007;19(1):22–6.
7. Jacobs I, Bast RC Jr. The CA 125 tumour-associated antigen: a review of the literature. Hum Reprod. 1989;4(1):1–12.
8. Sell S. Tumor Markers: Physiology, Pathobiology, Technology and Clinical Applications. Eleftherios P. Diamandis, Hervert A. Fritche, Hans Lilja, Daniel W. Chan, and Morton K. Schwartz, eds. Washington, DC: AACC Press, 2002, 513 pp., $99.00 ($79.00 AACC members), softcover. ISBN 1-890883-71-9. Clinical Chemistry. 2003;49(2):342.
9. Bast RC, Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB. CA 125: the past and the future. Int J Biol Markers. 1998;13(4):179–87.
10. 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.
11. Scholler N, Crawford M, Sato A, Drescher CW, O'Briant KC, Kiviat N, et al. Bead-based ELISA for validation of ovarian cancer early detection markers. Clin Cancer Res. 2006;12(7 Pt 1):2117–24.
12. Moore RG, Brown AK, Miller MC, Badgwell D, Lu Z, Allard WJ, et al. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. Gynecol Oncol. 2008;110(2):196–201.
13. Hellström 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.
14. Cviič D, Jagarlamudi K, Meglič L, Škof E, Zore A, Lukanović D, et al. A Dual Biomarker TK1 Protein and CA125 or HE4-Based Algorithm as a Better Diagnostic Tool than ROMA Index in Early Detection of Ovarian Cancer. Cancers. 2023;15(5):1593.
15. Samborski A, Miller MC, Blackman A, MacLaughlan-David S, Jackson A, Lambert-Messerlian G, et al. HE4 and CA125 serum biomarker monitoring in women with epithelial ovarian cancer. Tumour Biol. 2022;44(1):205–13.
16. 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. Obstet Gynecol. 2011;118(2 Pt 1):280–8.
17. Montagnana M, Danese E, Ruzzenente O, Bresciani V, Nuzzo T, Gelati M, et al. The ROMA (Risk of Ovarian Malignancy Algorithm) for estimating the risk of epithelial ovarian cancer in women presenting with pelvic mass: is it really useful? Clin Chem Lab Med. 2011;49(3):521–5.
18. Zhan Y, Chen J, Wu J, Gu Y, Huang Q, Deng Z, et al. Human epididymis protein 4 aggravates airway inflammation and remodeling in chronic obstructive pulmonary disease. Respir Res. 2022;23(1):120.
19. Sütő R, Pócsi M, Szabó Z, Fejes Z, Ivády G, Kerekes G, et al. Elevated level of serum human epididymis protein 4 (HE4) predicts disease severity and mortality in COVID-19 pneumonia. BMC Pulm Med. 2023;23(1):512.
20. Jacob F, Meier M, Caduff R, Goldstein D, Pochechueva T, Hacker N, et al. No benefit from combining HE4 and CA125 as ovarian tumor markers in a clinical setting. Gynecol Oncol. 2011;121(3):487–91.
21. Kallis GB, Holmgren A. Differential reactivity of the functional sulfhydryl groups of cysteine-32 and cysteine-35 present in the reduced form of thioredoxin from Escherichia coli. J Biol Chem. 1980;255(21):10261–5.
22. Park BJ, Cha MK, Kim IH. Thioredoxin 1 as a serum marker for ovarian cancer and its use in combination with CA125 for improving the sensitivity of ovarian cancer diagnoses. Biomarkers. 2014;19(7):604–10.
23. Cha MK, Park BJ, Kim IH. Thioredoxin1 as a novel serum marker for human ovarian cancer. FASEB J. 2012;26(S1):9675–9675.
24. Park BJ, Cha MK, Kim IH. Thioredoxin 1 as a serum marker for breast cancer and its use in combination with CEA or CA15-3 for improving the sensitivity of breast cancer diagnoses. BMC Res Notes. 2014;7:7.
25. Lee YJ, Kim Y, Choi BB, Kim JR, Ko HM, Suh KH, et al. The blood level of thioredoxin 1 as a supporting biomarker in the detection of breast cancer. BMC Cancer. 2022;22(1):12.
26. Shang W, Xie Z, Lu F, Fang D, Tang T, Bi R, et al. Increased Thioredoxin-1 Expression Promotes Cancer Progression and Predicts Poor Prognosis in Patients with Gastric Cancer. Oxid Med Cell Longev. 2019;2019:9291683.
27. Raffel J, Bhattacharyya AK, Gallegos A, Cui H, Einspahr JG, Alberts DS, et al. Increased expression of thioredoxin-1 in human colorectal cancer is associated with decreased patient survival. J Lab Clin Med. 2003;142(1):46–51.
28. Mandrekar JN. Receiver operating characteristic curve in diagnostic test assessment. J Thorac Oncol. 2010;5(9):1315–6.
29. Vickers AJ, Elkin EB. Decision curve analysis: a novel method for evaluating prediction models. Med Decis Mak. 2006;26:565–74.
30. Vickers AJ, van Calster B, Steyerberg EW. A simple, step-by-step guide to interpreting decision curve analysis. Diagn Progn Res. 2019;3:18.
31. Serov SF, Scully RE. Histological typing of ovarian tumours.

32. Zamwar UM, Anjankar AP, Aetiology. Epidemiology, Histopathology, Classification, Detailed Evaluation, and Treatment of Ovarian Cancer. Cureus. 2022;14(10):e30561.
33. Clauss A, Lilja H, Lundwall A. A locus on human chromosome 20 contains several genes expressing protease inhibitor domains with homology to whey acidic protein. Biochem J. 2002;368(Pt 1):233–42.
34. Li J, Dowdy S, Tipton T, Podratz K, Lu WG, Xie X, et al. HE4 as a biomarker for ovarian and endometrial cancer management. Expert Rev Mol Diagn. 2009;9(6):555–66.
35. Behrouzi R, Barr CE, Crosbie EJ. HE4 as a Biomarker for Endometrial Cancer. Cancers. 2021;13(19):4764.
36. Nagy B, Bhattoa HP, Steiber Z, Csobán M, Szilasi M, Méhes G, et al. Serum human epididymis protein 4 (HE4) as a tumor marker in men with lung cancer. Clin Chem Lab Med (CCLM). 2014;52(11):1639–48.
37. Small DM, Doherty DF, Dougan CM, Weldon S, Taggart CC. The role of whey acidic protein four-disulfide-core proteins in respiratory health and disease. Biol Chem. 2017;398(4):425–40.
38. Lai X, Kang M, Chen Y, Xu F, Wang K, Cao J. Elevated serum level of human epididymal protein 4 (HE4) predicts poor prognosis in the critically ill with sepsis: A prospective observational cohort study. Clin Biochem. 2022;109–110:79–85.
39. Luo J, Liang J, Wang S, Huang S, Zhou L, Shi Y, et al. Serum human epididymis secretory protein 4 correlates with sepsis-associated acute respiratory distress syndrome and 28-day mortality in critically ill patients. Ann Clin Biochem. 2022;59(5):338–46.
40. Schirinzi A, Cazzolla AP, Lovero R, Lo Muzio L, Testa NF, Ciavarella D, et al. New Insights in Laboratory Testing for COVID-19 Patients: Looking for the Role and Predictive Value of Human epididymis secretory protein 4 (HE4) and the Innate Immunity of the Oral Cavity and Respiratory Tract. Microorganisms. 2020;8(11):1718.
41. Wei X, Su J, Yang K, Wei J, Wan H, Cao X, et al. Elevations of serum cancer biomarkers correlate with severity of COVID-19. J Med Virol. 2020;92(10):2036–41.
42. Awan MUN, Yan F, Mahmood F, Bai L, Liu J, Bai J. The Functions of Thioredoxin 1 in Neurodegeneration. Antioxid Redox Signal. 2022;36(13–15):1023–36.
43. Griffiths HR, Bennett SJ, Olofsson P, Dunston CR. Thioredoxin as a putative biomarker and candidate target in age-related immune decline. Biochem Soc Trans. 2014;42(4):922–7.
44. Whayne TF, Parinandi N, Maulik N. Thioredoxins in cardiovascular disease. Can J Physiol Pharmacol. 2015;93(11):903–11.
45. Zeng XS, Geng WS, Chen L, Jia JJ. Thioredoxin as a Therapeutic Target in Cerebral Ischemia. Curr Pharm Des. 2018;24(25):2986–92.
46. Noike T, Miwa S, Soeda J, Kobayashi A, Miyagawa Sichi. Increased expression of thioredoxin-1, vascular endothelial growth factor, and redox factor-1 is associated with poor prognosis in patients with liver metastasis from colorectal cancer. Hum Pathol. 2008;39(2):201–8.
47. Guan X, Ning J, Fu W, Wang Y, Zhang J, Ding S. Helicobacter pylori with trx1 high expression promotes gastric diseases via upregulating the IL23A/NF-κB/IL8 pathway. Helicobacter. 2024;29(2):e13072.
48. Ribback S, Winter S, Klatte T, Schaeffeler E, Gellert M, Stühler V, et al. Thioredoxin 1 (Trx1) is associated with poor prognosis in clear cell renal cell carcinoma (ccRCC): an example for the crucial role of redox signaling in ccRCC. World J Urol. 2022;40(3):739–46.
49. Kakolyris S, Giatromanolaki A, Koukourakis M, Powis G, Souglakos J, Sivridis E, et al. Thioredoxin expression is associated with lymph node status and prognosis in early operable non-small cell lung cancer. Clin Cancer Res. 2001;7(10):3087–91.
50. 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. Gynecol Oncol. 2009;112(1):40–6.
51. Karlsen MA, Høgdall EVS, Christensen IJ, Borgfeldt C, Kalapotharakos G, Zdrazilova-Dubska L, et al. A novel diagnostic index combining HE4, CA125 and age may improve triage of women with suspected ovarian cancer - An international multicenter study in women with an ovarian mass. Gynecol Oncol. 2015;138(3):640–6.
52. Karlsen MA, Sandhu N, Høgdall C, Christensen IJ, Nedergaard L, Lundvall L, et al. Evaluation of HE4, CA125, risk of ovarian malignancy algorithm (ROMA) and risk of malignancy index (RMI) as diagnostic tools of epithelial ovarian cancer in patients with a pelvic mass. Gynecol Oncol. 2012;127(2):379–83.
53. Sandri MT, Bottari F, Franchi D, Boveri S, Candiani M, Ronzoni S, et al. Comparison of HE4, CA125 and ROMA algorithm in women with a pelvic mass: correlation with pathological outcome. Gynecol Oncol. 2013;128(2):233–8.
54. Zhang M, Zhao B, Xie J, Liang Y, Yang Z. Serum Human Epididymis Protein 4 is Associated with Renal Function and Diabetic Kidney Disease in Patients with Type 2 Diabetes Mellitus. Biomed Res Int. 2019;2019(1):4831459.
55. Nagy B, Krasznai ZT, Balla H, Csobán M, Antal-Szalmás P, Hernádi Z, et al. Elevated human epididymis protein 4 concentrations in chronic kidney disease. Ann Clin Biochem. 2012;49(Pt 4):377–80.
56. Yu Z, Nian C, Sun W, Liu X, Nian X. Elevated serum HE4 levels as a novel biomarker of disease severity and hepatic fibrosis in autoimmune hepatitis. Clin Chim Acta. 2024;559:119682.

57. Meng K, Tian M, Gui X, Xie M, Gao Y, Shi S, et al. Human epididymis protein 4 is associated with severity and poor prognosis of connective tissue disease-associated interstitial lung disease with usual interstitial pneumonia pattern. Int Immunopharmacol. 2022;108:108704.
58. Muri J, Thut H, Feng Q, Kopf M. Thioredoxin-1 distinctly promotes NF-κB target DNA binding and NLRP3 inflammasome activation independently of Txnip. Horng T, Rath S, editors. eLife. 2020;9:e53627.
59. Grogan TM, Fenoglio-Prieser C, Zeheb R, Bellamy W, Frutiger Y, Vela E, et al. Thioredoxin, a putative oncogene product, is overexpressed in gastric carcinoma and associated with increased proliferation and increased cell survival. Hum Pathol. 2000;31(4):475–81.
60. Ceccarelli J, Delfino L, Zappia E, Castellani P, Borghi M, Ferrini S, et al. The redox state of the lung cancer microenvironment depends on the levels of thioredoxin expressed by tumor cells and affects tumor progression and response to prooxidants. Int J Cancer. 2008;123(8):1770–8.
61. Bhatia M, McGrath KL, Di Trapani G, Charoentong P, Shah F, King MM, et al. The thioredoxin system in breast cancer cell invasion and migration. Redox Biol. 2016;8:68–78.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Authors and Affiliations

Mi A Park[1] · Songhak Kim[2] · Xiaoguang Yang[2] · Jong Am Song[2] · Geon Woo Lee[1] · Heon Jong Yoo[3,5] · Soyoung Kim[4] · Kyoung Hoon Suh[2] · Young Bok Ko[1,3]

✉ Kyoung Hoon Suh
one@ens-h.com

✉ Young Bok Ko
koyoung27@gmail.com

[1] Department of Obstetrics & Gynecology, Chungnam National University Hospital, 282, Munhwa-ro, Jung-gu, Daejeon, Republic of Korea

[2] E&S Healthcare, 42, Gukjegwahak 11-ro, Yuseong-gu, Daejeon, Republic of Korea

[3] Department of Obstetrics & Gynecology, Chungnam National University School of Medicine, 266, Munhwa-ro, Jung-gu, Daejeon, Republic of Korea

[4] Innovation Center for Industrial Mathematics, National Institute for Mathematical Sciences, 5 Seongnam, Korea

[5] Department of Obstetrics & Gynecology, Chungnam National University Sejong Hospital, 20, Bodeum 7-ro, Sejong, Republic of Korea