Evaluation of NID2 and CDO1 methylation for ovarian cancer screening

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Abstract. Ovarian cancer (OC) is a highly invasive disease with a poor prognosis, underscoring the importance of identifying specific biomarkers in early screening to enhance the overall survival of patients with OC. In this study, quantitative methylation-specific PCR was used to investigate the methylation levels of the nidogen-2 (NID2) and cysteine dioxygenase 1 (CDO1) gene promoters in peripheral blood samples from 72 patients with OC and 75 healthy individuals. The results revealed significantly higher methylation levels of the NID2 and CDO1 genes in patients with OC compared with those in healthy controls. NID2 methylation demonstrated a sensitivity of 70.83% and a specificity of 96.00% in predicting OC, whereas CDO1 exhibited a sensitivity of 90.28% and a specificity of 69.33%. The positivity rate of NID2 methylation was elevated in patients with stage III-IV OC compared with those with stage I-II OC and was higher in high-grade serous carcinoma compared with that in ovarian clear cell carcinoma. Additionally, the positivity rate of CDO1 methylation could reach 84.6% in patients with stage I-II OC. Furthermore, the methylation levels of NID2 and CDO1 exhibited a positive correlation with carbohydrate antigen 125 (CA125) levels. Combining the detection of NID2 and CDO1 methylation with CA125 significantly enhanced the sensitivity and specificity of OC detection compared with CA125 detection alone. In conclusion, enhanced methylation of the NID2 and CDO1 genes has emerged as an independent risk factor for OC development.

Introduction

Ovarian cancer (OC) is a prevalent malignancy affecting >320,000 women globally (1). Epithelial OC, constituting 90% of cases, is highly heterogeneous and poses challenges for early detection (2). The absence of sensitive diagnostic tools often leads to late-stage diagnoses, resulting in a low 5-year survival rate of 30% for advanced cases (3). Therefore, identifying precise biomarkers in early screening and predicting overall survival in high-risk OC populations is imperative to address the challenges of late-stage diagnosis and poor prognosis.

Carbohydrate antigen 125 (CA125) is a conventional tumor marker for OC, albeit with limited sensitivity (50-62%) and specificity (73-77%) (4). Elevated CA125 levels have been observed in pregnancy, pelvic inflammatory disease and various other types of cancer [including breast (5), uterine (6) and lung (7) cancer]. Previous research has highlighted the utility of cell-free DNA (cfDNA) methylation analysis in early cancer detection. Aberrant cfDNA methylation, detected in the initial stages of OC, modulates gene expression by methylating specific gene sites (8-10). For example, hypermethylation of the tumor suppressor genes homeobox A9 (HOXA9) and hypermethylated in cancer-zinc finger and BTB domain-containing protein transcriptional repressor 1 (HIC1) facilitates uncontrolled cell proliferation, contributing to OC development (11). Furthermore, hypermethylation of bromodomain-containing 4 enhances the invasive potential of OC (12). These gene methylation alterations represent promising biomarkers for early cancer diagnosis.

cfDNA harbors tumor-specific genetic alterations and can be utilized for non-invasive cancer detection by analyzing aberrant methylation patterns (13). Previous research has highlighted the significance of nidogen-2 (NID2) and cysteine dioxygenase 1 (CDO1) as tumor suppressor genes (14,15). NID2, a basement membrane component, serves a key role in maintaining membrane integrity, with its deficiency promoting cancer development (16). By contrast, upregulation of NID2 in lung cancer cells suppresses proliferation and invasiveness (17). CDO1 facilitates apoptosis by modulating antioxidant levels and is frequently epigenetically altered in various types of cancer, commonly through promoter methylation (18). Elevated CDO1 promoter methylation has been observed in solid tumors such as breast (19) and lung cancer (20). However, the methylation status of NID2 and CDO1 in OC remains poorly understood. The present study aimed to assess the methylation profiles of NID2 and CDO1 in OC, to establish associations with clinical data and to investigate their potential as specific biomarkers for OC screening.

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Patients and methods

Study population and blood sample collection. The present study recruited 147 participants who attended Hangzhou Cancer Hospital (Hangzhou, China) between February 2023 and June 2024. Among them, 72 were patients with a clear clinical diagnosis of OC and 75 were normal control (NC) group. Ovarian cancer staging was determined according to the International Federation of Gynecology and Obstetrics (FIGO) 2018 staging system (21). Inclusion criteria were as follows: i) Female patients with a confirmed diagnosis of OC; ii) aged between 35 and 75 years; iii) Eastern Cooperative Oncology Group (ECOG) performance status 0-4 (22); iv) hospitalized patients with a life expectancy of >2 months; v) participants voluntarily joined the present study and provided signed informed consent; and vi) patients were conscious and able to manage their own dietary control. Exclusion criteria were as follows: i) Expected hospital stay of <3 days; ii) underwent major surgery recently or any surgery lasting >2 h; iii) participation in any other clinical trial within 4 weeks prior to the start of the present study; iv) respiratory depression, (pulmonary) airway obstruction or tissue hypoxia; v) hematological diseases; vi) cardiac diseases [namely, cardiac function of New York Heart Association class II or higher (23)]; vii) pregnant or lactating women; viii) brain disorders with impaired judgment; and ix) unable to provide signed informed consent. A 1.5-ml peripheral blood sample was collected from each individual for methylation testing, with blood from patients with OC collected prior to antitumor therapy. Clinical data and the results of tumor marker CA125 detection were collected from all subjects. The cut-off value of CA125 was 35 U/ml. The present study was approved by the Institutional Review Board of Hangzhou Cancer Hospital (approval no. HZCH-2023-006; Hangzhou, China). Written informed consent to participate was obtained from all individual participants included in the present study prior to their enrolment. The present study was conducted in accordance with the principles of The Declaration of Helsinki.

DNA isolation and bisulfite conversion. cfDNA was extracted from serum samples using the QIAamp® DNA Mini Kit (Qiagen GmbH). The concentration and purity of the nucleic acids were measured using the NanoDrop™ One microvolume UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc.). For bisulfite conversion, 500 ng of the extracted cfDNA was used with the EZ DNA Methylation™ Kit (Zymo Research Corp.), and the reaction was carried out according to the manufacturer's protocol.

Quantitative methylation-specific PCR (qMSP). Methylation levels of the NID2 and CDO1 genes were analyzed using qMSP with the Applied Biosystems 7500 real-time PCR system (cat. no. ABI7500; Thermo Fisher Scientific, Inc.). Each reaction contained a fluorogenic probe labeled with VIC™ fluorescent dye (Thermo Fisher Scientific, Inc.) and conjugated with a minor groove binder. The primers were designed to adhere to the principle of sequence specificity following bisulfite conversion and pre-validated using PCR amplification using DNA templates with known methylation status that had undergone bisulfite treatment. The sequences of the primers used for qMSP are detailed in Table SI.

Each PCR was performed in a 96-well plate with β-actin as an internal reference gene and three parallel wells per reaction. Each assay rigorously incorporated both negative and positive controls. The thermal cycling conditions were as follows: i) 95˚C for 5 min; ii) 15 cycles at 95˚C for 15 sec and 64˚C for 30 sec; and iii) 30 cycles at 95˚C for 15 sec and 62˚C for 32 sec. For analysis, the quantification cycle (Cq) value for each sample was used, ΔCq was calculated using the following formula: ΔCq=average Cq target gene - average Cq internal reference. Gene methylation levels for each sample were expressed as $2^{-\Delta\Delta Cq}$, ΔΔCq=ΔCq (OC) - ΔCq (NC average) (24).

Statistical analysis. Statistical analyses were performed using SPSS (version 26.0; IBM Corp.). The Shapiro-Wilk test was employed to analyze the distribution pattern of the data. As the data exhibited significant dispersion, non-parametric tests were employed, and the results for each index were expressed as the median and interquartile range [M (25th percentile, 75th percentile)]. The Mann-Whitney U test was used to compare continuous variables between two groups. Spearman's correlation analysis was used to analyze correlations between variables. Categorical variables were expressed as n (%). The χ^2 test was used to compare count data. When the expected frequency in >20% of the cells was <5, Fisher's exact test was used instead. To adjust for potential confounding factors (hypertension and performance status), a propensity score matching (PSM) analysis was performed using a 1:1 nearest neighbor matching algorithm without replacement. Receiver operating characteristic (ROC) curve analysis was used to determine the best key value in predicting OC. Each qMSP assay was performed in triplicate, and the mean values were used for analysis. The χ^2 test and logistic regression analysis were used to determine the risk factors for OC. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics. The clinical baseline characteristics of the participants are presented in Table I. There were 72 individuals in the OC group, with a median age of 59.5 years, and the tissue typing was mainly high-grade serous carcinoma [HGSC; 88.9% (64/72)]. The majority of patients were in the advanced stages [81.9% (59/72)] of OC, with chemotherapy [88.9% (64/72)] being the primary treatment method. During hospitalization, disease progression occurred in 25/72 (34.7%) patients and 5/72 (6.9%) patients died. The NC group consisted of 75 healthy individuals, with a median age of 65.0 years. Analysis of CA125 levels in the two groups revealed that the median CA125 level in the OC group was 178.5 U/ml, whereas the median CA125 level in the NC group was 12.8 U/ml (normal reference value: <35 U/ml; $P<0.001$), with a statistically significant difference. Furthermore, patients with OC had a significantly higher prevalence of hypertension and worse performance status scores, whereas there was no significant difference in the prevalence of type 2 diabetes mellitus compared with that in healthy individuals.

Table I. Characteristics of participants in the present study.

Characteristics	NC (n=75)	OC (n=72)	P-value
Age, years [median (range)]	65.0 (55.0-71.0)	59.5 (54.0-66.8)	0.105
Histology			-
HGSC	-	64 (88.9)	
OCCC	-	8 (11.1)	
FIGO stage			-
I-II	-	13 (18.1)	
III-IV	-	59 (81.9)	
No metastasis	-	13 (18.1)	-
Metastasis			-
Lymph nodes	-	21 (29.2)	
Abdominal cavity	-	9 (12.5)	
Pelvis	-	14 (19.4)	
Liver	-	2 (2.8)	
Lungs	-	1 (1.4)	
Multiple metastases	-	12 (16.7)	
ECOG PS score			0.012
0-2	70 (93.3)	57 (79.2)	
3-4	5 (6.7)	15 (20.8)	
Hypertension	16 (21.3)	27 (37.5)	0.031
Type 2 diabetes mellitus	13 (17.3)	21 (29.2)	0.089
Family history of cancer	-	22 (30.6)	-
Treatment method			-
Chemotherapy	-	64 (88.9)	
Nutritional support	-	8 (11.1)	
CA125, U/ml (range)	12.8 (8.9-19.1)	178.5 (34.0-582.2)	<0.01
Primary therapy outcome			-
CR-PR	-	11 (15.3)	
SD	-	36 (50.0)	
PD	-	25 (34.7)	
Mortality	-	5 (6.9)	-

Values are expressed as n (%) unless otherwise specified. -, not applicable; NC, normal control; OC, ovarian cancer; HGSC, high-grade serous carcinoma; OCCC, ovarian clear cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics; ECOG PS, Eastern Cooperative Oncology Group performance status; CA125, carbohydrate antigen 125; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

DNA methylation levels of NID2 and CDO1 genes. Methylation levels were determined using qMSP (Fig. 1A and B). The results demonstrated that the methylation levels of the NID2 gene in the serum of the NC and OC groups were 0.41 (0.11-2.26) and $1.90x10^4$ ($1.49\text{-}7.75x10^4$), respectively, whereas the methylation levels of the CDO1 gene were 0.91 (0.01-180.1) and $2.83x10^3$ ($836.3\text{-}4.67x10^3$), respectively. Compared with the NC group, the methylation levels of NID2 and CDO1 in patients with OC were significantly higher (P<0.001). Furthermore, to evaluate the potential confounding effects of baseline hypertension status and physical fitness on methylation outcomes, PSM analysis was performed (Table SII; Fig. S1). Using a 1:1 nearest neighbor matching algorithm without replacement, 57 participants in the NC group were successfully matched to 57 patients in the OC group. Post-PSM analyses revealed

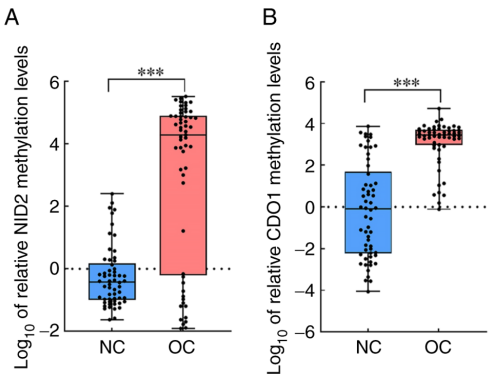

Figure 1. Measurement of (A) NID2 and (B) CDO1 DNA methylation levels in the serum of two groups of patients. DNA methylation levels are presented as $2^{-\Delta\Delta Cq}$. ***P<0.001. NC, normal control; OC, ovarian cancer; NID2, nidogen-2; CDO1, cysteine dioxygenase 1.

Table II. Diagnostic values of serum gene methylation makers for patients with ovarian cancer.

Markers	Cut-off	Sn, %	Sp, %	Youden index	AUC	P-value	95% CI
NID2	16.95	70.83	96.00	0.668	0.765	<0.001	0.677-0.853
CDO1	15.99	90.28	69.33	0.596	0.878	<0.001	0.880-0.965

NID2, nidogen-2; CDO1, cysteine dioxygenase 1; Sn, sensitivity; Sp, specificity; AUC, area under the curve.

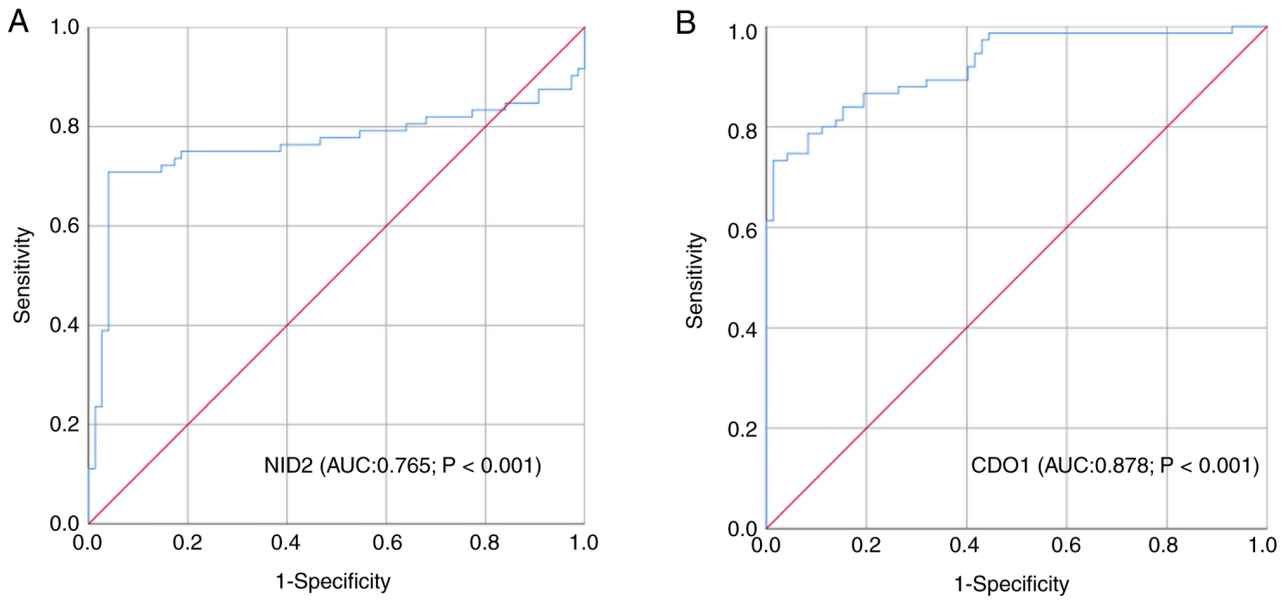

Figure 2. Diagnostic values of serum gene methylation makers for patients with ovarian cancer. (A) NID2 and (B) CDO1. NID2, nidogen-2; CDO1, cysteine dioxygenase 1; AUC, area under the curve.

that the methylation levels of both NID2 and CDO1 genes remained significantly elevated in the OC cohort compared with that in the matched controls (P<0.05), which is consistent with pre-matched observations.

Predictive value of NID2 and CDO1 gene DNA methylation for OC. To investigate the predictive value and detection cut-off values of the NID2 and CDO1 genes for OC, ROC curves were plotted using the ΔCq values. As presented in Fig. 2A and B, and Table II, the area under the curve (AUC) values in predicting OC with NID2 and CDO1 were 0.765 and 0.878, respectively. When the Youden index was maximized, the optimal ΔCq values in predicting OC with these markers were 16.95 for NID2 and 15.99 for CDO1. Among these, CDO1 exhibited a higher detection efficacy for OC, with an AUC of 0.878 (P<0.001).

Detection rates of the NID2 and CDO1 methylation test. Based on the cut-off values of NID2 and CDO1 gene methylation (Table II), the positive detection rates were calculated in different FIGO stages and pathological types (Fig. 3A-D; Table III). The results demonstrated that the positive detection rates of NID2 and CDO1 methylation in OC were 70.8% (51/72) and 90.3% (65/72), respectively. In stages I-II, the positive detection rates for NID2 and CDO1 methylation were 46.2% (6/13) and 84.6% (11/13), respectively, and in stages III-IV, the positive detection rates were 76.3% (45/59) and 91.5% (54/59), respectively. Furthermore, the positive detection rates of NID2 and CDO1 methylation in HGSC were 71.9% (46/64) and 90.6% (58/64), respectively, whereas in ovarian clear cell carcinoma, the positive detection rates were 62.5% (5/8) and 87.5% (7/8), respectively. The clinical data analysis indicated that patients with OC ≥65 years of age and with FIGO stages III-IV had a higher positive detection rate for NID2 methylation, with statistically significant differences (both P<0.05), whereas there were no significant differences in different histological subtypes and various prognoses among the patients. Additionally, no significant changes were observed in the positive detection rate of CDO1 methylation among these variables (all P>0.05).

Combined detection of NID2 and CDO1 gene DNA methylation and CA125. To further evaluate the predictive value of NID2 and CDO1 gene DNA methylation for OC, the serum levels of the traditional OC marker CA125 were also analyzed (Fig. 4A). The results indicated that the serum CA125 level in the NC group was 12.8 (8.90-19.10) U/ml, whereas in the OC group it was 178.45 (34.00-582.23) U/ml, which was significantly higher compared with that in the NC group (P<0.01). Correlation analysis demonstrated that serum CA125 was positively correlated with NID2 (r=0.341; P<0.0001) and CDO1 (r=0.527; P<0.0001) (Fig. 4B and C). Furthermore,

Table III. Detection rates of NID2 and CDO1 methylation.

	NID2 (n=72)			CDO1 (n=72)		
Characteristics	Negative (n=21)	Positive (n=51)	P-value	Negative (n=7)	Positive (n=65)	P-value
Age, years			0.015			0.413
≥65	18 (85.7)	27 (52.9)		3 (42.9)	42 (64.6)	
<65	3 (14.3)	24 (47.1)		4 (57.1)	23 (35.4)	
FIGO stage			0.031			0.602
I-II	7 (33.3)	6 (11.8)		2 (28.6)	11 (16.9)	
III-IV	14 (66.7)	45 (88.2)		5 (71.4)	54 (83.1)	
Histology			0.684			0.578
HGSC	18 (85.7)	46 (90.2)		6 (85.7)	58 (89.2)	
OCCC	3 (14.3)	5 (9.8)		1 (14.3)	7 (10.8)	
Prognosis			0.495			>0.999
DCB	15 (71.4)	32 (62.7)		5 (71.4)	42 (64.6)	
Non-DCB	6 (28.6)	19 (37.3)		2 (28.6)	23 (35.4)	

Values are expressed as n (%). NID2, nidogen-2; CDO1, cysteine dioxygenase 1; FIGO, International Federation of Gynecology and Obstetrics; HGSC, high-grade serous carcinoma; OCCC, ovarian clear cell carcinoma; DCB, durable clinical benefit.

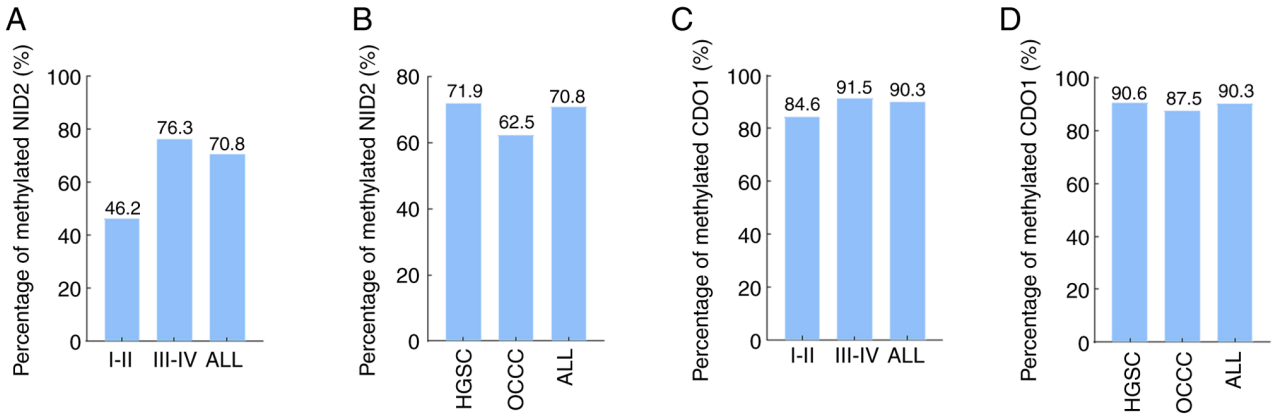

Figure 3. Positive detection rates of NID2 in (A) different FIGO stages and (B) different pathological types. Positive detection rates of CDO1 in (C) different FIGO stages and (D) different pathological types. FIGO, International Federation of Gynecology and Obstetrics; NID2, nidogen-2; CDO1, cysteine dioxygenase 1; HGSC, high-grade serous carcinoma; OCCC, ovarian clear cell carcinoma; ALL, all cases (combined total).

ROC curves for the three markers across different FIGO stages of OC demonstrated that the AUC values of NID2 and CDO1 methylation, and CA125 were 0.560 (P=0.491), 0.916 (P<0.001) and 0.893 (P<0.001), respectively, in patients with FIGO stage I-II OC (Fig. S2; Table SIII). Notably, CDO1 methylation exhibited the highest sensitivity (84.6%), whereas CA125 exhibited the highest specificity (97.3%). In patients with stage III-IV OC (Fig. S2; Table SIV), the AUC values were 0.810 (P<0.001), 0.870 (P<0.001) and 0.878 (P<0.001), respectively, with CDO1 methylation achieving the highest sensitivity (91.5%) and NID2 methylation demonstrating the highest specificity (96.0%). The three markers were further arranged and combined, and according to the ROC curve results, among the multiple combined detections, the combination of CA125 + NID2 + CDO1 exhibited the highest efficacy, with an AUC value of 0.971 (P<0.001), a sensitivity of 86.1% and a specificity of 97.3% (Fig. 4D; Table IV).

Risk factor analysis for the occurrence of OC. The present study combined the predictive serum levels of NID2, CDO1 and CA125 for OC with clinical data to perform a risk factor analysis (Fig. 5). The results indicated that serum CA125 level >31.05 U/ml [odds ratio (OR), 118.09; 95% CI, 26.182-532.619; P<0.001], NID2 ΔCq <16.95 (OR, 58.286; 95% CI, 16.504-205.848; P<0.001) and CDO1 ΔCq <15.99 (OR, 20.994; 95% CI, 8.358-52.747; P<0.001) are potential risk factors for the occurrence of OC.

Discussion

DNA methylation is a pivotal early event in cancer initiation, offering potential for cancer diagnosis and therapy. Recent studies have highlighted the key involvement of DNA methylation in the onset (25), progression (26) and drug resistance (27) of OC. Serum methylation markers exhibited promise in

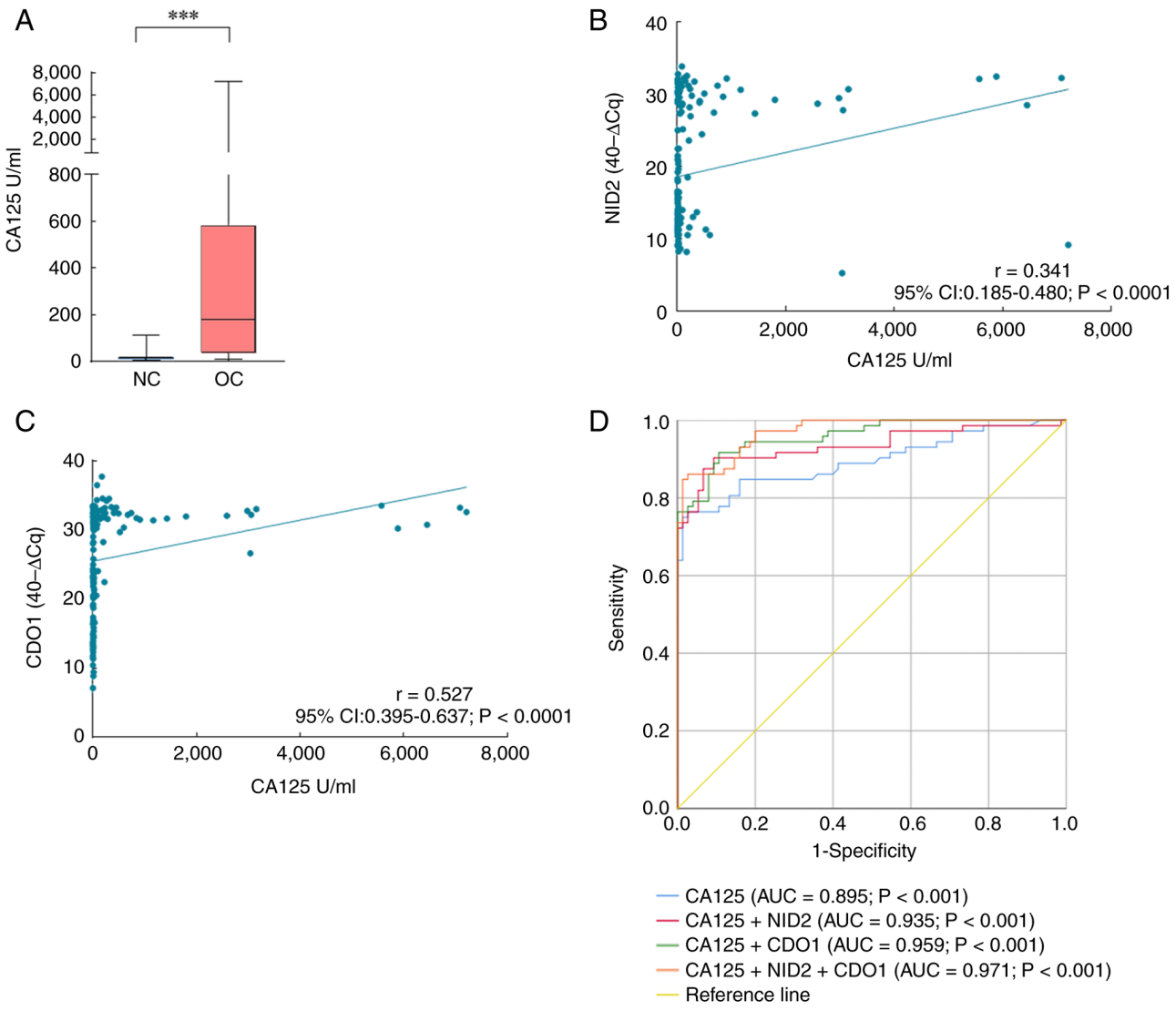

Figure 4. Evaluation of CA125, NID2 and CDO1 in ovarian cancer. (A) Serum CA125 levels. Correlation analyses between (B) NID2 and CA125, and (C) CDO1 and CA125. (D) Receiver operating characteristic curves for the prediction of OC with CA125, NID2 and CDO1. ***P<0.001. CA125, carbohydrate antigen 125; NID2, nidogen-2; CDO1, cysteine dioxygenase 1; AUC, area under the curve; NC, normal control; OC, ovarian cancer.

detecting OC within the initial 2 years of diagnosis, enabling early detection and personalized treatment strategies for OC (28,29).

The present study revealed that patients with OC exhibited elevated methylation levels of NID2 and CDO1 compared with healthy individuals. This finding was further validated in the post-PSM analyses, confirming the robustness of the observed association. Specifically, older patients with advanced-stage OC exhibited a higher prevalence of NID2 methylation, whereas its efficacy was diminished in early-stage OC. This may be associated with the case cohort that was primarily composed of patients with late-stage disease. However, since the majority of patients with OC are already in the advanced stages of the disease at their first clinical visit, the results of methylation testing in recent years have also been generally influenced by late-stage bias, with markedly reduced sensitivity in early asymptomatic patients (30-32). Notably, in the present study, the positive rate of CDO1 methylation did not demonstrate a significant difference across different FIGO stages, and CDO1 methylation demonstrated high detection sensitivity in patients with both early-stage (84.6%) and advanced-stage (91.5%) OC. These findings indicated that CDO1 methylation may become an important marker for the early screening of OC. Despite exhibiting significantly lower diagnostic capability in early-stage OC compared with that in late-stage OC, NID2 methylation maintained consistently higher detection specificity compared with CDO1 (96.00%) throughout the analysis.

Compared with existing diagnostic methods, CA125, currently relied upon for OC diagnosis, has insufficient sensitivity and specificity in early-stage disease and certain pathological types, while imaging techniques are insensitive to microscopic lesions (33). In the present study, CDO1 methylation maintained a high positive rate (84.6%; 11/13) and strong discriminatory ability (AUC=0.916) even in patients with FIGO stage I-II, suggesting its potential to address the shortcomings of current methods in early screening. Prior research has demonstrated that integrating methylation data from multiple genes can markedly enhance the accuracy of OC detection in comparison with the utilization of individual

Table IV. Predictive value of combined detection of NID2 and CDO1 gene DNA methylation and CA125 for ovarian cancer.

Markers	Sn, %	Sp, %	Youden index	AUC	P-value	95% CI
CA125	76.4	97.3	0.737	0.895	<0.001	0.840-0.949
CA125 + NID2	90.3	90.7	0.809	0.935	<0.001	0.891-0.979
CA125 + CDO1	91.7	89.3	0.810	0.959	<0.001	0.931-0.987
CA125 + NID2 + CDO1	86.1	97.3	0.834	0.971	<0.001	0.950-0.992

NID2, nidogen-2; CDO1, cysteine dioxygenase 1; CA125, carbohydrate antigen 125; Sn, sensitivity; Sp, specificity; AUC, area under the curve.

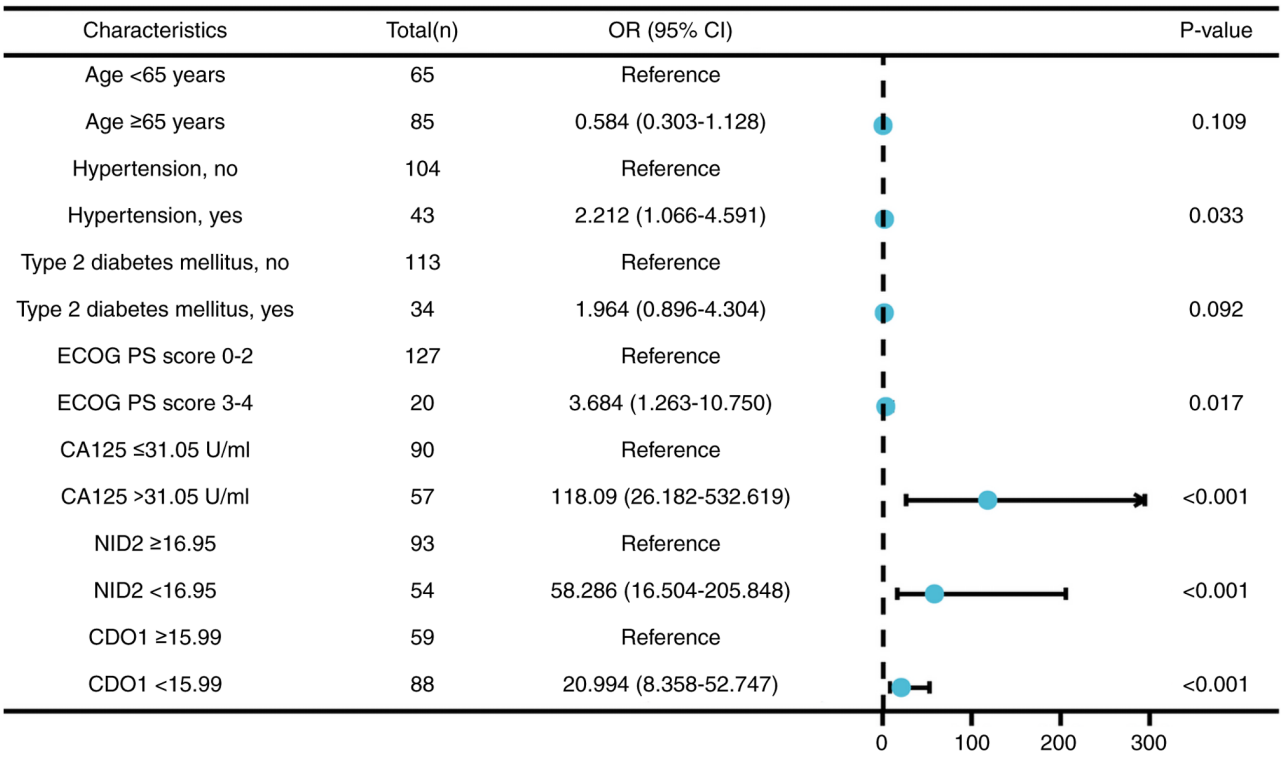

Figure 5. Risk factor analysis for the occurrence of ovarian cancer. CA125, carbohydrate antigen 125; NID2, nidogen-2; CDO1, cysteine dioxygenase 1; ECOG PS, Eastern Cooperative Oncology Group performance status; OR, odds ratio.

genes (34,35). A previous meta-analysis focusing on OC revealed that out of 16 methylated genes, the sensitivity for OC detection varied from 41.4 to 100%, with 12 genes exhibiting a sensitivity of >75%. Furthermore, the specificity for OC detection ranged from 55.0 to 100%, with seven genes demonstrating a specificity of >95% (30). The present study revealed a significantly positive correlation between the methylation levels of NID2 and CDO1 and the conventional OC serological marker CA125. The combined assay of the three detection markers further improved diagnostic sensitivity (86.1%) and specificity (97.3%) for OC, thereby partially addressing the limitations in sensitivity and specificity associated with CA125 as a single biomarker. However, compared with mature biomarker detection systems, methylation testing currently incurs higher costs and requires standardized experimental procedures and threshold definition, which presents a challenge for its broader clinical translation in the future (36).

The clinical utility of NID2 and CDO1 as methylation biomarkers requires evaluation within the broader context of methylation screening technologies. As a key tumor suppressor gene, promoter hypermethylation of CDO1 is a common epigenetic event in various solid tumors, including breast (19), endometrial (37) and lung cancer (38). This aligns biologically with the notable hypermethylation observed in the serum of patients with OC in the present study. More notably, recent whole-genome sequencing studies have identified CDO1 hypermethylation in both urine and tissue samples from patients with OC (39). The present study has further confirmed this phenomenon in blood samples and demonstrated its diagnostic performance using quantitative analysis, collectively reinforcing the scientific foundation and translational potential of CDO1 hypermethylation as a cross-cancer biomarker for OC. In contrast to CDO1, although direct evidence for NID2 promoter methylation in OC cfDNA is limited, NID2, as a basement membrane-associated gene,

has been repeatedly reported to exhibit promoter hypermethylation and functional silencing in other malignancies such as gastrointestinal (40) and lung cancer (17). Mechanistic studies have demonstrated that NID2 hypermethylation leads to reduced expression, while demethylation or upregulation can inhibit tumor cell proliferation, migration and invasion, supporting the hypothesis that NID2 may act as a tumor suppressor silenced via methylation (17,41,42). In the field of OC, previous research has largely focused on NID2 protein levels, revealing elevated serum levels associated with CA125 (43,44). The present study findings further confirmed that NID2 promoter hypermethylation can be detected in serum cfDNA with high specificity, providing novel evidence from an epigenetic perspective in understanding its involvement in OC development and progression (44,45). In a recent study on OC epigenetics, Faaborg *et al* (46) detected HOXA9 gene methylation in 93% (82/88) of patients with OC. In addition, Singh *et al* (11) developed a novel epigenetic panel (combining HOXA9 and HIC1 methylation) achieving 88.9% sensitivity with an AUC value of 0.950. The combined detection model assessed in the present study demonstrated comparable performance to these extensively researched epigenetic panels, with a sensitivity of 86.1%, a specificity of 97.3% and an AUC value of 0.971.

Enhancing early screening and diagnosis of patients with OC may improve clinical outcomes and decrease mortality rates. Liquid biopsy has emerged as a viable option for broad early screening, with higher patient acceptability compared with tissue biopsy due to its minimally invasive nature (47,48). Previous studies have indicated that cfDNA methylation assay outcomes are typically consistent with tumor tissue methylation assay results and encompass a notable portion of solid tumor genetic data, rendering the findings of the present study clinically applicable (4,49,50). In addition, cfDNA methylation could potentially associate with treatment outcomes and prognosis in patients (51). In a previous study analyzing data from 2,636 participants across 15 studies, Kalachand *et al* (52) observed that BRCA1 methylation displayed similar clinicopathological characteristics to BRCA1 mutations in patients with OC. However, the predictive capability of BRCA1 methylation for platinum sensitivity and survival prognosis remains to be elucidated. Furthermore, Tserpeli *et al* (53) observed that the predictive capacity for platinum resistance in OC could be evaluated via Schlafen family member 11 methylation, an aspect that warrants further exploration.

To the best of our knowledge, the novelty of the present study is reflected in the evaluation of NID2 promoter methylation in serum cfDNA from patients with OC, which demonstrated its complementary diagnostic utility to CDO1, and in the development of a clinically applicable multi-marker model that combines these methylation markers with CA125. Several limitations of the present study warrant consideration. Firstly, the present study predominantly comprised patients with advanced-stage OC, necessitating further prospective studies to evaluate the predictive potential of NID2 and CDO1 in early-stage OC. The clinical translation of the present findings warrants validation through multi-center collaborations that recruit larger cohorts of patients with FIGO stage I-II OC alongside comparative groups, including benign ovarian lesions and high-risk populations, with longitudinal monitoring of NID2 and CDO1 methylation dynamics to establish their early-warning potential. Furthermore, the methylation cut-off values determined by the Youden index in the present study require prospective validation in broader populations to confirm generalizability. Another key limitation lies in the suboptimal specificity of CDO1 methylation, necessitating systematic characterization of its methylation kinetics in non-malignant conditions to establish disease-specific thresholds. Integrating these thresholds with traditional imaging modalities could optimize diagnostic accuracy, targeting the high sensitivity of CDO1 for primary screening. Furthermore, its utility in recurrence monitoring warrants investigation.

In conclusion, the present study indicated that the methylation status of NID2 and CDO1 may function as serological biomarkers for the detection and identification of OC and could have potential clinical significance in the future.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

MJ conceived the present study. SW carried out the design of the present study. SW, YC and JJ performed the experiments. JJ and QH collected clinical samples. SW, YC, QH and XH analyzed the data and wrote the manuscript. SW and MJ cconfirm the authenticity of all the raw data. MJ and XH revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of Hangzhou Cancer Hospital (approval no. HZCH-2023-006; Hangzhou, China). Written informed consent to participate was obtained from all individual participants included in the present study prior to their enrolment. The present study was conducted in accordance with the principles of the Declaration of Helsinki.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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