Research Article

Serum Autoantibodies against LRDD, STC1, and FOXA1 as Biomarkers in the Detection of Ovarian Cancer

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Purpose. This study is aimed at evaluating serum autoantibodies against four tumor-associated antigens, including LRDD, STC1, FOXA1, and EDNRB, as biomarkers in the immunodiagnosis of ovarian cancer (OC).

Methods. The autoantibodies against LRDD, STC1, FOXA1, and EDNRB were measured using an enzyme-linked immunosorbent assay (ELISA) in 94 OC patients and 94 normal healthy controls (NHC) in the research group. In addition, the diagnostic values of different autoantibodies were validated in another independent validation group, which comprised 136 OC patients, 136 NHC, and 181 patients with benign ovarian diseases (BOD).

Results. In the research group, autoantibodies against LRDD, STC1, and FOXA1 had higher serum titer in OC patients than NHC (P < 0.001). The area under receiver operating characteristic curves (AUCs) of these three autoantibodies were 0.910, 0.879, and 0.817, respectively. In the validation group, they showed AUCs of 0.759, 0.762, and 0.817 and sensitivities of 49.3%, 42.7%, and 48.5%, respectively, at specificity over 90% for discriminating OC patients from NHC. For discriminating OC patients from BOD, they showed AUCs of 0.718, 0.729, and 0.814 and sensitivities of 47.1%, 39.0%, and 51.5%, respectively, at specificity over 90%. The parallel analyses demonstrated that the combination of anti-LRDD and anti-FOXA1 autoantibodies achieved the optimal diagnostic performance with the sensitivity of 58.1% at 87.5% specificity and accuracy of 72.8%. The positive rate of the optimal autoantibody panel improved from 62.4% to 87.1% when combined with CA125 in detecting OC patients.

Conclusion. Serum autoantibodies against LRDD, STC1, and FOXA1 have potential diagnostic values in detecting OC.

1. Introduction

Ovarian cancer (OC) remains the deadliest cancer in women worldwide. There were an estimated 22,530 new cases and 13,980 deaths from OC in the United States in 2019, making it the leading cause of cancer deaths among gynecologic malignancies [1]. Although advances in OC treatment significantly improved the five-year survival of OC patients over the last three decades, the overall cure rate remained less than 30% [2]. When the lesion is restricted within the ovaries, up to 90% of patients can be cured following routine surgery and chemotherapy, and the five-year survival rate is approximately 50% for disease limit to the pelvis (stage II) with treatment strategies [3]. Meanwhile, the five-year survival rate for the disease beyond the pelvis (stage III-IV) is less than 20% [4]. However, due to the absence of specific symptoms at the early stage, the vast majority of OC patients were diagnosed at an advanced stage (stage III or stage IV), and only 20% of OC patients were initially diagnosed at an early stage [5]. Hence, the quest for applicable and reliable
biomarkers for early detection is critical in improving the clinical outcomes of OC patients.

Carbohydrate antigen 125 (CA125) and transvaginal ultrasonography (TVUs) are typically used in clinical settings and can detect some OC patients at early stage. Human epididymal protein (HE4) can detect a small fraction of OC patients missed by CA125 [3]. The study showed that the diagnostic value of CA125 and HE4 with areas under the receiver operating characteristic curves (AUCs) were 0.78 and 0.76, respectively, for the discrimination between benign and stage I OC [6]. However, these serum markers are not ideal biomarkers in the early detection of OC due to their limited sensitivity [7]. TVUs lack adequate sensitivity and yield a high false-positive rate [8, 9]. Therefore, the discovery of biomarkers for the early detection of OC is of considerable importance. Tumor-associated antigens (TAAs) are a kind of protein aberrantly expressed in cancer, which can elicit an autoimmune response and the production of corresponding autoantibodies accordingly [10]. Autoantibodies against TAAs are more stable and have a relatively higher titer in serum or plasma due to the amplification effect of the immune system compared with their corresponding TAAs [11].

LRDD, also known as PIDD, which contains two protein interaction domains, is a leucine-rich repeat and death domain-containing protein [12]. It is induced by TP53 and acts as a molecular switch to promote cell survival or apoptosis [13]. A previous study showed that LRDD was the downstream target gene of TP53, and it could increase the TP53 protein expression in a positive feedback loop [14, 15]. Stanniocalcin-1 (STC1) is a glycoprotein hormone, and it is involved in regulating calcium and phosphate homeostasis, which was initially discovered in bony fishes [16]. Mammalian STC1 is expressed in various tissues, with the highest levels in the ovary, prostate, kidney, lung, colon, and thyroid [17, 18]. Studies show that STC1 functions as a proto-oncogene and participates in the biological process of tumorigenesis [19]. Forkhead-box A1 (FOXa1), also known as hepatocyte nuclear factor 3-a (HNF3a), is a central regulator in the normal development of several endoderm-derived tissues [20]. FOXA1 can directly bind to the DNA and open the chromatin to enhance the transcription; so, it is described as a pioneer transcription activator [21]. It has been confirmed that FOXA1 is positively expressed in OC tissue, and it is involved in the pathogenesis and development of OC [22]. Endothelin receptor type B (EDNRB) is a member of the family of G-protein-coupled receptors, which plays a vital role in tumor cell proliferation, migration, and lymph angiogenesis via combination with endothelin-1 [23, 24]. Aberrant methylation of EDNRB and decreased expression of mRNA were identified in various cancers [25].

There is growing evidence that these four proteins are associated with the occurrence and development of cancer. However, studies on the feasibility that their corresponding autoantibodies serve as biomarkers of cancer are still sparse, especially for OC. Therefore, this study is aimed at evaluating the diagnostic value of their corresponding autoantibodies in OC detection.

2. Materials and Methods

2.1. Serum Samples. The research group comprised sera from 94 OC patients and 94 normal healthy controls (NHC), respectively. Sera from OC patients were obtained from a tertiary level hospital (Zhengzhou, China) from March 1, 2011, to April 30, 2012, and sera of NHC were collected from the cardiovascular disease investigation project (Henan Province, China) without the benign ovarian disease (BOD) or disease associated with the immune system. The validation group comprised 453 sera from 136 OC patients with histological confirmation, 136 NHC, and 181 patients with BOD. In the validation group, sera from OC and BOD patients were obtained from a tertiary level hospital (Zhengzhou, China) from July 1, 2017, to April 30, 2018, and sera from NHC was derived from the biobank of Henan Key Laboratory of Tumor Epidemiology. The collection of all serum samples followed standardized protocol, and serum samples were stored at −80°C until further use. All patients signed written informed consent, and the current study was approved by the Institutional Review Board of Zhengzhou University.

2.2. Enzyme-Linked Immunosorbent Assay (ELISA). Autoantibodies against LRDD, STC1, EDNRB, and FOXA1 in human serum samples were measured by ELISA, and the detailed protocol were described previously [26]. In brief, three recombinant proteins (Cloud-Clone, China) were diluted to the optimal concentration (0.125 μg/mL, respectively) using a coating buffer. Recombinant proteins (50 μl/well) were added to the 96-well ELISA plates, incubated at 4°C overnight. 96-well plates were blocked with 2% bovine serum albumin (BSA) at 4°C overnight to reduce the non-specific reaction. Then, PBST (0.01% Tween 20 in phosphate-buffered saline) was used to wash the plates three times. Next, human serum samples diluted at 1:100 in 1% BSA were added to the antigen-coated 96-well plates. The plates were incubated at 37°C for 1 hour. Following by five times of wash with PBST, the secondary antibody goat anti-human IgG horseradish peroxidase-conjugated (HRP) diluted at 1:5000 was added to each well for 1 hour incubated at 37°C followed by washing five times with PBST. The solution of 3,3′,5,5′-tetramethylbenzidine (TMB)-H₂O₂-urea was used as detecting reagents. 25 μL of 2 M sulfuric acid served as the stopping solution. The optical density (OD) values were measured at 450 nm and 620 nm with a multimode plate reader (PerkinElmer envision 2105, USA). For quality control, six fixed human serum samples were used as references to mitigate batch effects between plates, and the last two wells of the last column of each plate served as blank controls, respectively.

2.3. Statistical Analysis. The differences of autoantibodies between OC and NHC were assessed using independent sample t-test and Mann-Whitney U test. One-way analysis of variance (ANOVA) and the Kruskal-Wallis H test were applied to compare the differences in more than two groups. The cutoff values (the OD value corresponding to the maximal Youden index at specificity over 90%) were used to determine a positive reaction for each autoantibody. The
Chi-square test was conducted to compare the positive rates between the OC group and NHC group. The receiver operating characteristic (ROC), sensitivity, specificity, negative likelihood ratio ($LR^-$), positive likelihood ratio ($LR^+$), and accuracy rate were performed to assess the diagnostic performance of each autoantibody. Two-tailed $P$ values less than 0.05 were considered statistically different. Statistical analysis was carried out by SPSS 26.0 and GraphPad prism software 8.0.

3. Results

3.1. Characteristics of the Study Population. In the present study, two independent groups were designed to investigate the diagnostic values of autoantibodies against 4 TAAs for OC in 641 serum samples by ELISA. The research group enrolled 94 OC patients with age of $54.2 \pm 12.0$ years (range from 21 to 83 years) and 94 NHC with age of $56.9 \pm 12.7$ years (range from 27 to 83 years). The validation group included 136 OC patients with age of $51.9 \pm 11.9$ years (range from 16 to 74 years), 136 NHC with age of $50.2 \pm 11.6$ years (range from 20 to 83 years), and 181 BOD patients with age of $35.8 \pm 10.6$ years (range from 20 to 68 years). Table 1 shows the clinicopathological features of the study population for both research and validation groups.

|            | Research group | Validation group |
|------------|----------------|------------------|
|            | OC ($n = 94$)  | NHC ($n = 94$)   | OC ($n = 136$)  | NHC ($n = 136$)  | BOD ($n = 181$) |
| Age (year) |               |                  |                |                  |                |
| Range      | 21-83         | 27-83            | 16-74          | 20-83            | 20-68          |
| Mean ± SD  | 54.2 ± 12.0   | 56.9 ± 12.7      | 51.9 ± 11.9    | 50.2 ± 11.6      | 35.8 ± 10.6 |
| Gender     | Female        | Female           | Female         | Female           | Female         |
| FIGO stage |               |                  |                |                  |                |
| I-II       | 3 (3.2)       | 33 (24.3)        |                |                  |                |
| III-IV     | 48 (51.1)     | 77 (56.6)        |                |                  |                |
| Unknown    | 43 (45.7)     | 26 (19.1)        |                |                  |                |
| FIGO stage |               |                  |                |                  |                |
| I-II       | 55 (58.5)     | 111 (81.6)       |                |                  |                |
| III-IV     | 3 (3.19)      | 9 (6.6)          |                |                  |                |
| Unknown    | 35 (37.2)     | 11 (8.1)         |                |                  |                |
| FIGO stage |               |                  |                |                  |                |
| I-II       | 6 (6.4)       | 48 (35.3)        |                |                  |                |
| III-IV     | 14 (14.9)     | 49 (36.0)        |                |                  |                |
| Unknown    | 74 (78.7)     | 39 (28.7)        |                |                  |                |
| FIGO stage |               |                  |                |                  |                |
| I-II       | 40 (42.6)     | 35 (25.7)        |                |                  |                |
| III-IV     | 14 (14.9)     | 50 (36.8)        |                |                  |                |
| Unknown    | 40 (42.6)     | 51 (37.5)        |                |                  |                |
| FIGO stage |               |                  |                |                  |                |
| I-II       | 4 (4.3)       | 19 (14.0)        |                |                  |                |
| III-IV     | 4 (4.3)       | 19 (14.0)        |                |                  |                |
| Unknown    | 74 (78.7)     | 39 (28.7)        |                |                  |                |
| FIGO stage |               |                  |                |                  |                |
| I-II       | 6 (6.4)       | 48 (35.3)        |                |                  |                |
| III-IV     | 14 (14.9)     | 49 (36.0)        |                |                  |                |
| Unknown    | 74 (78.7)     | 39 (28.7)        |                |                  |                |
| FIGO stage |               |                  |                |                  |                |
| I-II       | 40 (42.6)     | 35 (25.7)        |                |                  |                |
| III-IV     | 14 (14.9)     | 50 (36.8)        |                |                  |                |
| Unknown    | 40 (42.6)     | 51 (37.5)        |                |                  |                |

Table 1: The clinicopathological features of the study population for both research and validation group.

Abbreviations: OC: ovarian cancer; NHC: normal healthy controls; BOD: benign ovarian diseases.
autoantibodies were measured in another independent group of 136 OC patients, 136 NHC, and 181 BOD patients. The median levels and interquartile ranges of serum autoantibodies in the research and validation group are present in Table 2. Serum autoantibodies against LRDD, STC1, and FOXA1 significantly increased in OC patients compared to NHC and BOD patients (\( P < 0.001 \)). The serum titer of anti-LRDD autoantibody was higher in BOD patients than that in NHC (\( P < 0.05 \)), while no statistical differences were found between BOD patients and NHC for anti-STC1 and anti-FOXA1 autoantibodies (\( P > 0.05 \)) (Figure 1(b)). Subsequently, we performed the ROC curves to evaluate the diagnostic values of these three autoantibodies in the validation group. The AUCs of anti-LRDD, STC1, and FOXA1 autoantibodies were 0.759, 0.762, and 0.819, respectively (Figure 3). When BOD patients were used as controls, serum autoantibodies against LRDD, STC1, and FOXA1 showed AUCs of 0.718, 0.729, and 0.814 in discriminating OC and BOD patients, respectively (Figure 3). A significant difference was found between NHC and early or late-stage OC for anti-LRDD, STC1, and FOXA1 autoantibodies (\( P < 0.001 \), Figure 4). The potential diagnostic performance of the three
autoantibodies was further assessed in different stages of OC. None of the AUCs showed a significant difference between early-stage and late-stage OC (Figure 4).

3.4. Positive Rates of Anti-LRDD, STC1, and FOXA1 Autoantibodies in OC and NHC. The OD value corresponding to the maximal Youden index at specificity over 90% was used as the cutoff value to determine a positive reaction for each autoantibody. As shown in Table 3, the sensitivities of anti-LRDD, STC1, and FOXA1 autoantibodies were 79.79%, 65.96%, and 44.68%, respectively, in the research group. In the validation group, the sensitivities of anti-LRDD, STC1, and FOXA1 autoantibodies were 49.26%, 47.1%, and 44.68%, respectively, in the research group. As shown in Table 4, the sensitivities increased to 59.56% with successive addition of all the three autoantibodies, and the specificity slightly dropped from 90.44% to 87.50%. The maximum YI and accuracy rate from a panel comprising anti-LRDD and anti-FOXA1 autoantibodies reached 0.46 and 72.79%, respectively.

3.5. Association between Positive Rates of Autoantibodies and Clinical Characteristics of OC Patients. To compare the positive rates of autoantibody between OC patients with different clinical characteristics, OC patients were classified into different subgroups according to age, family history of tumor, TNM stage, tumor size, lymph node metastasis, and distant metastasis. As shown in Table 5, the positive rate of anti-LRDD autoantibody in OC patients without distant metastasis was significantly higher than patients with distant metastasis ($P < 0.05$). The positive rates appeared to be slightly higher in OC patients at late-stage and with a family history of cancer than OC patients at an early stage and without a family history of tumor, respectively.
3.6. Combination of Optimal Autoantibody Panel and CA125 in OC Detection. Eighty-five out of 136 patients in the research group had detailed information on CA125. The positive rates of CA125 and the optimal autoantibody panel alone were 60.0% and 62.4% in OC patients, respectively. However, when we combined the optimal autoantibodies panel with CA125, the positive rate in OC patients increased to 87.1% (Table 6). Further analysis revealed that the combination of optimal autoantibody panel and CA125 was significantly superior to the CA125 or the autoantibody panel alone in detecting OC ($P < 0.001$).

4. Discussion

The improvement of survival outcomes in OC is substantially determined by the timely diagnosis and appropriate treatment [27, 28]. To date, the early detection of OC has
Table 3: Diagnostic value of autoantibody against LRDD, STC1, FOXA1, and EDNRB in human sera by ELISA in the research and validation group.

| TAAb       | Se (%) | Sp (%) | YI | LR+  | LR-  | PPV (%) | NPV (%) | Accuracy (%) |
|------------|--------|--------|----|------|------|---------|---------|--------------|
| **Research group** |
| LRDD      | 79.79  | 90.43  | 0.70 | 8.33 | 0.22 | 89.29   | 81.73   | 85.11        |
| STC1      | 65.96  | 91.49  | 0.57 | 7.75 | 0.37 | 88.57   | 72.88   | 78.72        |
| FOXA1     | 44.68  | 90.43  | 0.35 | 4.67 | 0.61 | 82.35   | 62.04   | 67.55        |
| EDNRB     | 12.77  | 91.49  | 0.04 | 1.5  | 0.95 | 60.00   | 51.19   | 52.13        |
| **Validation group** |
| LRDD      | 49.26  | 90.44  | 0.40 | 5.15 | 0.56 | 83.75   | 64.06   | 69.85        |
| STC1      | 42.65  | 91.91  | 0.35 | 5.27 | 0.62 | 84.06   | 61.58   | 67.28        |
| FOXA1     | 48.53  | 91.18  | 0.40 | 5.50 | 0.56 | 84.62   | 63.92   | 69.85        |

Abbreviations: OC: ovarian cancer; NHC: normal healthy controls; Se: sensitivity; Sp: specificity; YI: Youden index; LR+: positive likelihood ratio; LR-: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value.

Table 4: Diagnostic value of the combinations of autoantibodies.

| Panel of TAAbs | Positive, No. (%) | Se, (%) | Sp (%) | YI | LR+  | LR-  | PPV (%) | NPV, (%) | Accuracy (%) |
|---------------|------------------|---------|--------|----|------|------|---------|----------|--------------|
| **OC (n = 136)** | **NHC (n = 136)** |
| LRDD          | 67 (49.26)       | 49.26   | 90.44  | 0.40 | 5.15 | 0.56 | 83.75   | 64.06    | 69.85        |
| LRDD, FOXA1   | 79 (58.09)       | 58.09   | 87.50  | 0.46 | 4.65 | 0.48 | 82.30   | 67.61    | 72.79        |
| LRDD, FOXA1, STC1 | 81 (59.56)     | 59.56   | 85.29  | 0.45 | 4.05 | 0.47 | 80.20   | 67.84    | 72.43        |

Abbreviations: OC: ovarian cancer; NHC: normal healthy controls; Se: sensitivity; Sp: specificity; YI: Youden index; LR+: positive likelihood ratio; LR-: negative likelihood ratio; PPV: positive predictive value; NPV: negative predictive value.

Table 5: Subgroup analysis of autoantibody level and clinical characteristics of OC patients.

| Variables                      | n | Anti-LRDD | Anti-STC1 | Anti-FOXA1 | Anti-LRDD or anti-FOXA1 |
|-------------------------------|---|-----------|-----------|------------|------------------------|
|                               |   | Positive (%) | Positive (%) | Positive (%) | Positive (%) |
| Age (year)                    |   |            |            |            |            |
| <50                           | 52 | 23 (44.2)  | 22 (42.3)  | 27 (51.9)  | 29 (55.8)   |
| ≥50                           | 84 | 43 (51.2)  | 35 (41.7)  | 38 (45.2)  | 50 (59.5)   |
| Family history of tumor       |   |            |            |            |            |
| No                            | 81 | 39 (48.1)  | 34 (42.0)  | 39 (48.1)  | 48 (59.3)   |
| Yes                           | 36 | 20 (55.6)  | 18 (50.0)  | 20 (55.6)  | 23 (63.9)   |
| TNM stage                     |   |            |            |            |            |
| Early stage (I + II)          | 34 | 17 (50.0)  | 13 (38.2)  | 15 (44.1)  | 19 (55.9)   |
| Late stage (III + IV)         | 76 | 39 (51.3)  | 36 (47.4)  | 40 (52.6)  | 46 (60.5)   |
| Tumor size                    |   |            |            |            |            |
| <5 cm                         | 9  | 4 (44.4)   | 5 (44.4)   | 5 (55.6)   | 5 (55.6)    |
| ≥5 cm                         | 33 | 15 (45.5)  | 13 (39.4)  | 14 (42.4)  | 18 (54.5)   |
| Lymph node metastasis         |   |            |            |            |            |
| Positive                      | 49 | 23 (46.9)  | 21 (42.9)  | 25 (51.0)  | 29 (59.2)   |
| Negative                      | 48 | 23 (47.9)  | 18 (37.5)  | 22 (45.8)  | 27 (56.3)   |
| Distant metastasis            |   |            |            |            |            |
| No                            | 35 | 20 (57.1)  | 15 (42.9)  | 19 (54.3)  | 24 (68.6)   |
| Yes                           | 50 | 17 (34.0)  | 18 (36.0)  | 19 (38.0)  | 26 (52.0)   |

Disease Markers
been hindered by the paucity of effective serum biomarkers. Previous studies have shown that the elevation of anti-TP53 autoantibody levels provided the first biomarker lead time over CA125 to diagnose preclinical diseases in a fraction of cases [29]. This study evaluated the diagnostic value of three biomarkers, including anti-LRDD, anti-STC1, and anti-FOX1 autoantibodies, in detecting OC. The sensitivity ranged from 42.65% to 49.26% for individual autoantibody at above 90% specificity. The serum levels of these three autoantibodies were significantly higher in OC patients than that in NHC and BOD patients. Besides, the AUC ranged from 0.759 to 0.819, which indicated that autoantibodies from 0.759 to 0.819, which indicated that autoantibodies targeting LRDD, STC1, and FOXA1 could be used as biomarkers in detecting OC. An optimal panel of anti-LRDD and anti-FOX1 autoantibodies was identified. The addition of this autoantibody panel to CA125 achieved a higher positive rate in detecting OC than the use of CA125 or the panel of two autoantibodies alone.

Tumorigenesis is a complex process that involves multigenic alterations [30]. Oncogenic transformation is due in part to the accumulation of DNA damage [15]. LRDD serves a prominent role in response to DNA damage by mediating the transcription factor NF-κappa-B (NF-κB) activation [31]. Berube and colleagues confirmed that the expression of LRDD could be detected in the nuclear and cytoplasmic fractions of mouse and human cell lines, function as the component of the DNA damage, or genotoxic stress response pathway [14]. Bradley et al. found that the expression of LRDD showed a wide range of oral squamous cell carcinoma, and the expression is extremely high in the tumor with p53 mutation [32]. Accumulating evidence presented that the broad expression of LRDD can be detected in non-small-cell lung cancer tissues [33]. There was a correlation between the high expression of LRDD and clinical data, such as tumor size, tumor stage, and lymph node metastasis. Patients with the high LRDD expression were associated with poor survival [33]. Mounting researches indicated that the higher expression of STC1 was associated with OC, breast cancer, and hepatocellular carcinoma [34–36]. It is noteworthy that the roles of STC1 are intricate in OC and breast cancer. The occurrence of OC and breast cancer is related to the down-regulation of STC1 after losing BRCA1 function [37, 38]. It has been shown that STC1 played a crucial role in promoting tumor metastasis, invasion, tumor cell proliferation, and antiapoptosis via participating in multiple signal pathways associated with cancer, including JNK/Jun NF-κB, cyclin E/CDK-2, and ERK1/2 signal pathways [39–42]. Besides, STC1 also could enhance tumor angiogenesis via activation of the VEGF/VEGFR-2 signaling pathway [43]. One study suggested that STC1 was positively regulated by desumoylated progesterone receptor in the absence of ligand for the breast cancer cells [37]. It has been demonstrated that STC1 exhibited significant clinical value in the diagnosis, prognosis, and pathological parameters for many kinds of cancer patients [43]. Therefore, STC1 holds promise as a biomarker in the early diagnosis of cancer. FOX1 is regarded as an oncogene that involves in the tumorigenesis and progression of hormone-dependent cancers [44]. Meanwhile, FOXA1 also has a tumor-suppressive function by suppressing the PI3K signaling pathway, a potential cancer therapeutic target [45]. Mutations in the FOXA1 gene have been recurrently reported in prostate cancer, ER-positive breast cancer, and liver cancer [44, 46, 47]. Increasing studies substantiated that the FOXA1 overexpression can promote tumor metastasis, invasion, and proliferation, particularly in several hormone-independent cancers [47]. In salivary duct carcinoma and bladder cancer, patients with high FOXA1 expression levels are associated with favorable clinical survival outcomes [48, 49]. Data available in immunohistochemical studies suggested that the expression of FOXA1 in OC and salivary duct carcinoma tissue is significantly higher than that in normal tissue, and it may be a potential biomarker for cancer detection [48, 50]. Although these three proteins were proven to have an important effect on the onset of cancer, few studies have investigated the values of these proteins as biomarkers in the diagnosis of cancer.

In the current study, this is the first time to evaluate the diagnostic value of anti-LRDD, anti-STC1, and anti-FOX1 autoantibodies in OC. There is a significant difference between the OC and the two control groups (NHC and BOD groups). However, the sensitivity for a single autoantibody is limited at over 90% specificity. Therefore, the combinational utilization of autoantibodies could enhance the sensitivity without significantly compromising the specificity. Notably, the parallel combination of anti-LRDD and anti-FOX1 autoantibodies achieved a sensitivity of 58.09% at the specificity of 87.50%, and the accuracy rate was 72.79%. Wang et al. analyzed 132 OC and 147 NHC, and they showed the sensitivity of 61.4% at the specificity of 85.0% in OC by the parallel combination of nine autoantibodies (autoantibodies against p53, c-MYC, p90, p62, AHSG, and 14-3-3 zeta, RalA, Koc, P16) [51]. Li et al. reported that a panel comprising nine autoantibodies against survivin, p53, p16, cyclin B1, cyclin D1, cyclin A, cyclin E, Koc, and IMP1, P62, CDK2, P90, and c-MYC achieved a sensitivity of 62.5% at 85.4% specificity in the detection of OC [52]. In our study, the panel comprising two autoantibodies was more cost-effective than the panels from the two studies mentioned above. Besides, the results from these two studies have been constrained by lacking an independent validation group compared to our present study.

We also examined the positive rates of autoantibody between different subgroups divided by different clinical characteristics. There was no significant relationship between positive rates of autoantibody and clinical characteristics in OC patients. This may be due to the limited serum samples and incomplete patient data used in this study. Therefore,
in the later rounds of the study, there is a need to expand the sample size and collect more detailed clinical characteristics of the cases for further exploring. For most OC patients presenting at a late stage at the time of diagnosis, clinical blood specimens before the diagnosis were unavailable. So, the main limitation of this study is that it was a retrospective study. We focus solely on the identification and validation phase of biomarkers. Further large-scale prospective investigations are required to confirm the diagnostic values of these autoantibodies.

In conclusion, this study indicates that anti-LRDD, anti-STC1, and anti-FOXA1 autoantibodies have high diagnostic values and may complement other serological biomarkers for OC detection. The combination of anti-LRDD and anti-FOXA1 autoantibodies acquired higher sensitivity of detection in OC patients. The combinational utilization of CA125 and anti-LRDD, anti-FOXA1 autoantibodies is promising in detecting OC in the clinical setting. However, the combination of autoantibodies remains to be investigated before future clinical implementation.

Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethical Approval

The study was approved by the Institutional Review Board of Zhengzhou University.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Authors’ Contributions

YD analyzed the data, prepared figures and tables, and authored the paper. CC, CQ, GS, XW, PW, HY, and LD checked the data. JS conceived and designed the research. All authors reviewed drafts of the paper, revised the manuscript, and approved the publication of the current manuscript.

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