Serum levels of alpha1-antitrypsin isoforms in patients with ovarian clear cell carcinoma: An exploratory study

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Abstract

Background: Ovarian clear cell carcinoma (OCCC) is frequently associated with endometriosis. Since serum levels of cancer antigen 125 (CA125) have limited diagnostic and prognostic value in this malignancy, there is an unmet need for reliable and specific biomarkers. Previous findings indicated that alpha 1-antitrypsin isoforms (isoAAT) are significantly increased in the peritoneal fluid of patients with endometriosis. This study was undertaken to examine whether serum isoAAT levels in patients with OCCC differ from those measured in women with endometriosis or benign ovarian tumors. We also investigated whether this biomarker may be useful for predicting survival in OCCC.

Methods: Paired serum samples before and after debulking surgery were collected from 27 patients with OCCC. All sera from patients with endometriosis (n = 44) and benign ovarian tumors (n = 32) were obtained in the pretreatment phase. Serum isoAAT levels were assayed using a proprietary ELISA kit.

Results: The highest levels of serum isoAAT (median, range) were identified in patients with OCCC (preoperative values: 160.9 ng/mL, range, 101.4–1098.8 ng/mL), followed by patients with endometriosis (125.0 and 83.4–473.2 ng/mL), and those with benign tumors (125.2 and 60.5–191.3 ng/mL). The differences in serum isoAAT levels between patients with OCCC and benign tumors were significant ($p = 0.041$). Debulking surgery of OCCC resulted in a significant decrease in serum isoAAT levels compared with the preoperative period (median, 160.9 versus 113.0 ng/mL, respectively, $p = 0.012$). As for prognostic prediction, we found that none of the nine patients with OCCC and serum isoAAT levels ≤130 ng/mL died of disease.

Conclusion: Serum isoAAT levels may be diagnostically useful to distinguish OCCC from benign ovarian tumors and could also serve as a potential prognostic marker.

Keywords: Alpha 1-antitrypsin isoforms; Biomarker; Diagnosis; Ovarian clear cell carcinoma; Survival

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Conflicts of interest: Dr Yang is an employee of V-CHECK (Zhubei City, Taiwan). The ELISA kit described in this study is manufactured by V-CHECK and is protected by a patent (US 9,229,012 B2). The other authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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1. INTRODUCTION

While being rare in Western countries, ovarian clear cell carcinoma (OCCC) poses a significant health burden in Asian women.[1] Endometriosis—a common gynecological condition characterized by the presence of endometrial tissue outside the uterine cavity[2]—is frequently associated with and considered to be a risk factor for OCCC.[3] Women with OCCC are more likely to be diagnosed at earlier stages than those with ovarian high-grade serous carcinoma, but their clinical outcomes continue to remain less favorable.[4,5] On CT images, OCCC typically appears as a unilocular, cystic mass with smooth margins and a size of 2–30 cm.[6] While large tumors may partly protrude in the lumen and be visualized under contrast enhancement, small-sized protruding lesions can be missed. The lack of reliable and specific biomarkers to facilitate screening and prompt diagnosis also eventually leads to poor prognosis. While serum levels of cancer antigen 125 (CA125) can be clinically useful in the preoperative discrimination between benign and malignant ovarian tumors,[7]

the next steps in improving their diagnostic performance in OCCC might involve combining different markers.[8]

Alpha 1-antitrypsin (AAT)—the most abundant circulating serine proteinase inhibitor—is mainly synthesized in the liver.[9] Iso-AAT (isoAAT) is an isoform of AAT. The variability of AAT glycosylation results in different isoforms with distinct isoelectric points.[10,11] The molecular weight of isoAAT ranges between 58 and 72 kDa, whereas that of AAT is approximately 55 kDa.[12] Notably, isoAAT is the result of posttranslational modifications of native AAT.[11] While isoAAT levels are increased in the peritoneal fluid of patients with endometriosis,[12,13] their diagnostic and prognostic value in OCCC—an endometriosis-associated malignancy—remains to be elucidated.

The aim of the present exploratory study was three-fold. First, we sought to examine whether serum isoAAT levels in patients with OCCC differ from those measured in women with endometriosis or benign ovarian tumors. Second, we analyzed the associations between serum isoAAT levels and the clinicopathological characteristics of patients with OCCC. Finally, we examined whether this new biomarker may have prognostic implications in this malignancy.

2. METHODS

2.1. Sample collection

This study was conducted using data obtained from patients with OCCC (n = 27) and endometriosis (n = 44) who were treated between January 2014 and October 2020. Patients with benign ovarian tumors (mucinous cystadenomas; n = 32) who underwent surgery served as controls. Paired serum samples before and after debulking surgery were collected from patients with OCCC. Conversely, all sera from patients with endometriosis and benign ovarian tumors were obtained in the pretreatment phase. The study protocol conforms to the tenets of the Helsinki Declaration and ethics approval was received from the local Institutional Review Board (approval number: 202001898B0). Owing to the retrospective nature of the study, the need for informed consent was waived.

2.2. Quantification of serum isoAAT levels

Serum samples (5 mL) were collected from all participants and stored at −80°C until analysis. Serum IsoAAT levels were measured using a commercially available enzyme-linked immunosorbent assay (Human isoAAT ELISA kit; V-CHECK, Inc., Zhubei City, Taiwan) according to the manufacturer's instructions. Briefly, serum samples (50 μL) and diluted standards were placed in a 96-well polystyrene plate and incubated for 1 hour at 30°C. After washing three times with a buffer solution (300 μL), the plate was placed on a horizontal shaker at 70–100 rpm for 5 min. After incubation with the primary antibody (50 μL) for 1 hour at 30°C, wells were washed and subsequently incubated with tetramethylbenzidine solution (50 μL) at 30°C in a dark room. When significant color changes were observed (required time: 30 min), a stop solution was added (50 μL) and optical density (OD) was measured at 450 nm on a Victor 2 microplate reader (PerkinElmer, Boston, MA, USA).

2.3. Immunohistochemistry

Immunohistochemistry (IHC) was performed using OCCC and benign ovarian mucinous cystadenoma specimens. Formalin-fixed, paraffin-embedded tissue sections (thickness: 4 μm thick) were stained with a mouse antihuman isoAAT monoclonal antibody (1:100 dilution; V-CHECK, Inc., Zhubei City, Taiwan) on an automated IHC stainer (BOND-MAX; Leica Biosystems, Nussloch, Germany) according to the manufacturer's protocol.

2.4. Data collection and outcome definition

Variables collected from the study participants were age at diagnosis, International Federation of Gynecology and Obstetrics (FIGO) stage, results of surgical debulking, presence of ascites, and serum CA125 levels. Cancer-specific survival (CSS) was defined as the time from diagnosis to OCCC-related death. Progression-free survival (PFS) was calculated as the time elapsed from the date of surgery to the date of documented disease progression, which was retrospectively defined according to imaging findings and/or increased serum CA125 levels.

2.5. Statistical analysis

We compared serum isoAAT concentrations among the three study groups using the Kruskal–Wallis test followed by Dunn's tests. Patients with OCCC were compared on preoperative versus postoperative serum isoAAT levels using Wilcoxon signed-rank tests. The optimal cutoff value of isoAAT to discriminate between OCCC patients who survived versus those who died of disease was identified with receiver operating characteristic (ROC) curve analysis. Kaplan–Meier estimate curves for CSS and PFS were generated and survival differences were compared with the log-rank test. Patients with OCCC were followed until they died or the date of last follow-up, whichever occurred first. All analyses were conducted using SPSS, version 19.0 (IBM, Armonk, NY, USA). Statistical significance was determined by a two-tailed p value <0.05.

3. RESULTS

3.1. Patient characteristics

The characteristics of the 27 patients with OCCC are summarized in the Table. According to the FIGO staging system, early- and late-stage OCCC was diagnosed in 15 and 12 patients, respectively. Three patients in this group had been treated with neoadjuvant chemotherapy before surgery.

3.2. Serum isoAAT levels in the three study groups

On IHC, isoAAT was found to be expressed in OCCC specimens (Fig. 1A). The calibration curve of isoAAT levels versus the measured OD was characterized by a linear slope (r^2 = 0.996;

Table 1

Characteristics of patients with ovarian clear cell carcinoma (n = 27) in relation to serum isoAAT levels

Characteristic	n (%)	Serum isoAAT levels, ng/mL, median (range)	p
Age, y, median (range)	49.8 (37.4, 69.8)	160.9 (101.4, 1098.8)	
Age, y			0.627
<50	14 (51.9)	134.6 (104.3, 1098.8)	
≥50	13 (48.1)	173.2 (101.4, 309.0)	
FIGO stage			0.192
I	12 (44.4)	125.3 (101.4, 1098.8)	
II	3 (11.1)	160.9 (107.6, 186.6)	
III	8 (29.6)	192.6 (133.3, 477.2)	
IV	4 (14.8)	134.5 (105.4, 173.2)	
Surgical debulking			0.891
Optimal	23 (85.2)	160.9 (101.4, 1098.8)	
Suboptimal	4 (14.8)	160.4 (133.0, 191.0)	
Presence of ascites			0.348
No	11 (40.7)	126.2 (101.4, 1098.8)	
Yes	16 (59.3)	167.3 (105.4, 477.2)	
Pretreatment serum CA125 levels, U/mL			0.029
<35	5 (18.5)	124.5 (101.4, 161.5)	
≥35	22 (81.5)	179.9 (105.4, 1098.8)	

CA125 = cancer antigen 125; FIGO = International Federation of Gynecology and Obstetrics; isoAAT = alpha 1-antitrypsin isoforms.

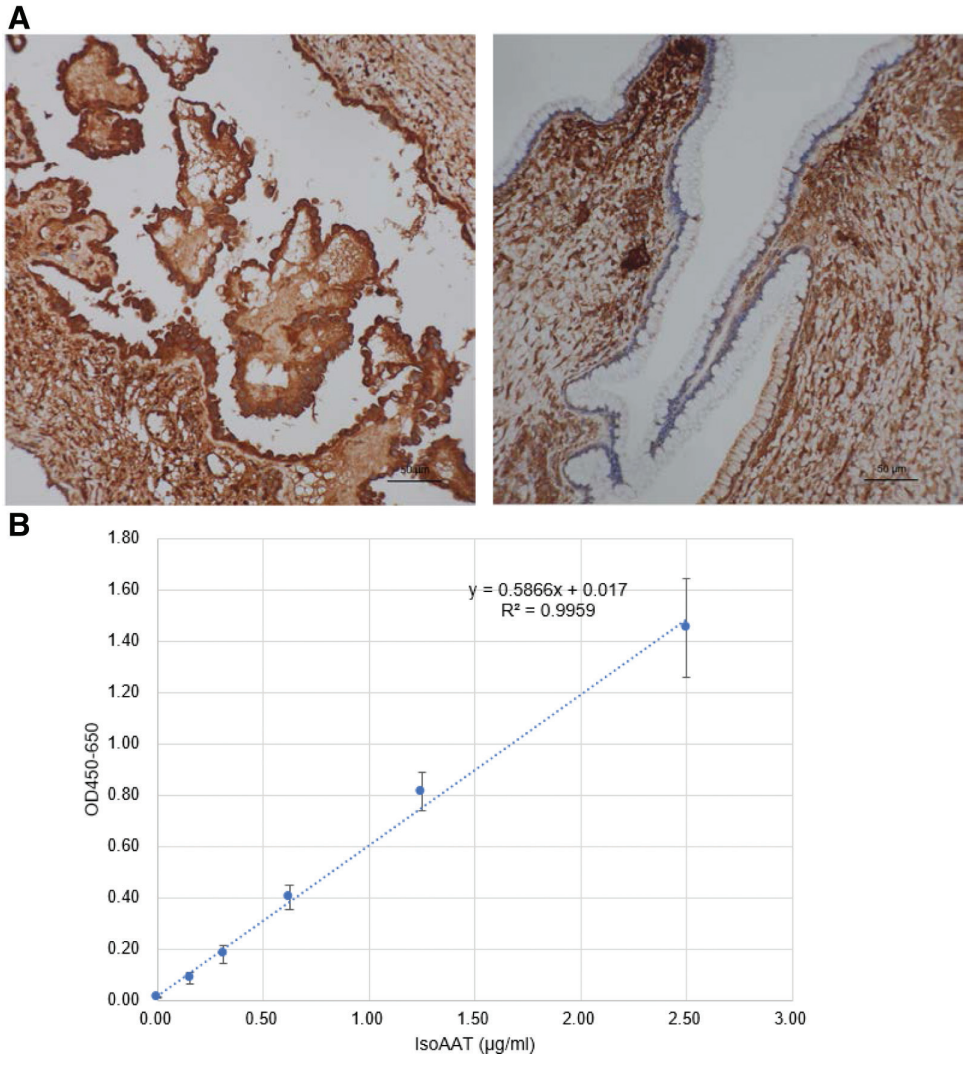

Fig. 1 Immunohistochemistry of isoAAT in human ovarian clear cell carcinoma (OCCC) and calibration curve of the isoAAT enzyme-linked immunosorbent assay kit used in the current study. A, Immunohistochemical expression of isoAAT in OCCC (left panel) and mucinous ovarian cystadenoma (right panel) specimens. Slides were counterstained with hematoxylin, and the photography was taken with a 20× objective lens. Only faint immunostaining was evident in benign mucinous cystadenoma specimens. B, The linear range of the standard (analytical range) was 0–2.5 μg/mL. isoAAT = alpha 1-antitrypsin isoforms; OCCC = ovarian clear cell carcinoma.

Fig. 1B). The highest levels (median, range) of serum isoAAT were identified in patients with OCCC (preoperative values: 160.9 ng/mL, range, 101.4–1098.8 ng/mL), followed by patients with endometriosis (125.0 and 83.4–473.2 ng/mL) and those with benign tumors (125.2 and 60.5–191.3 ng/mL). Using the Kruskal–Wallis test followed by Bonferroni adjustment based on Dunn's test, we found significant differences (p = 0.044) in serum isoAAT levels among the three study groups. While the difference in serum isoAAT levels between patients with OCCC and benign tumors was statistically significant (p = 0.041; Fig. 2), no significant differences between patients with OCCC and endometriosis was observed (p = 0.831).

3.3. Differences in serum isoAAT levels before and after debulking surgery in patients with OCCC

We next examined the impact of debulking surgery on isoAAT levels in patients with OCCC. We observed a significant decrease (p = 0.012) from preoperative (160.9 ng/mL, range, 101.4–1098.8 ng/mL) to postoperative (113.0 and 96.8–398.4 ng/mL) concentrations (Fig. 3). Compared with the preoperative values, the postoperative levels of isoAAT were found to be decreased in 19 of the 27 patients with OCCC.

3.4. Serum isoAAT concentrations in relation with the clinicopathological characteristics of patients with OCCC

We found no significant associations between serum isoAAT concentrations and the clinicopathological characteristics of patients with OCCC, including age, FIGO stage, results of surgical debulking, and presence of ascites (Table). On analyzing serum isoAAT concentrations in relation to CA125, we found that higher serum isoAAT levels were significantly associated with higher CA125 concentrations.

3.5. Prognostic significance of serum isoAAT concentrations in patients with OCCC

A total of 27 patients with OCCC were included in survival analyses. The median duration of follow-up for this patient group was 36.8 months (range: 2.1–75.4 months). Kaplan–Meier curves according to the optimal cut-off value for serum isoAAT (130 ng/mL, Fig. 4A) revealed a trend towards a lower

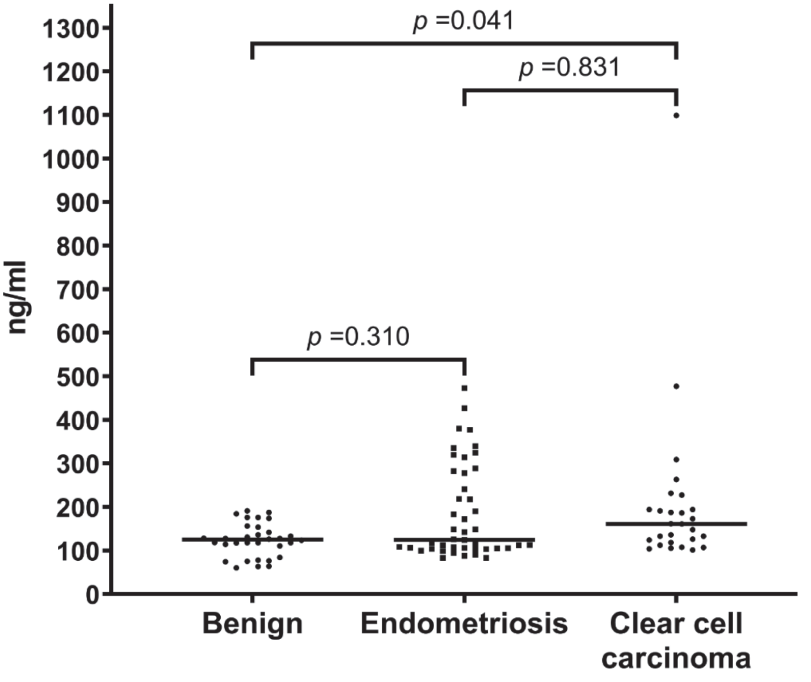

Fig. 2 Serum isoAAT levels in patients with OCCC (n = 27), endometriosis (n = 44), and benign ovarian tumors (n = 32). Horizontal lines indicate median values. isoAAT = alpha 1-antitrypsin isoforms; OCCC = ovarian clear cell carcinoma.

CSS in patients with increased isoAAT concentrations, albeit not significant (p = 0.068; Fig. 4B). None of the nine patients with isoAAT ≤130 ng/mL died of disease; thus, the 2- and 5-year CSS rates for these patients were both 100%. The 2- and 5-year CSS rates for patients with isoAAT >130 ng/mL were 71.1% and 59.2%, respectively. We did not find any significant association between serum isoAAT levels and PFS (Fig. 4C). No significant association between CA125 levels and CSS was observed in our cohort. However, PFS was marginally less favorable in patients with CA125 >35 U/mL than in those with CA125 ≤35 U/mL (Fig. 4D and E).

4. DISCUSSION

This is, to our knowledge, the first study to investigate the potential clinical utility of serum isoAAT levels in differentiating OCCC from benign ovarian tumors and endometriosis, as well as the first to evaluate their prognostic significance in patients with OCCC. We found that serum isoAAT levels were significantly higher in OCCC than in benign ovarian tumors, whereas no significant differences were observed between OCCC and endometriosis. We also demonstrated that debulking surgery resulted in a significant decrease in serum isoAAT levels compared with the preoperative period. As for prognostic prediction, we found that none of the nine patients with OCCC and serum isoAAT levels ≤130 ng/mL died of disease. While we did not identify significant associations between serum isoAAT levels and the clinical characteristics of patients with OCCC, our results demonstrate that this biomarker holds promise as a diagnostic and prognostic tool in this ovarian malignancy.

AAT, which is a protein encoded by the SERPINA1 gene, has been shown to protect against cellular damage induced by proteolytic enzymes.[14] Under physiological conditions, AAT is produced in the liver. However, increased AAT levels may be

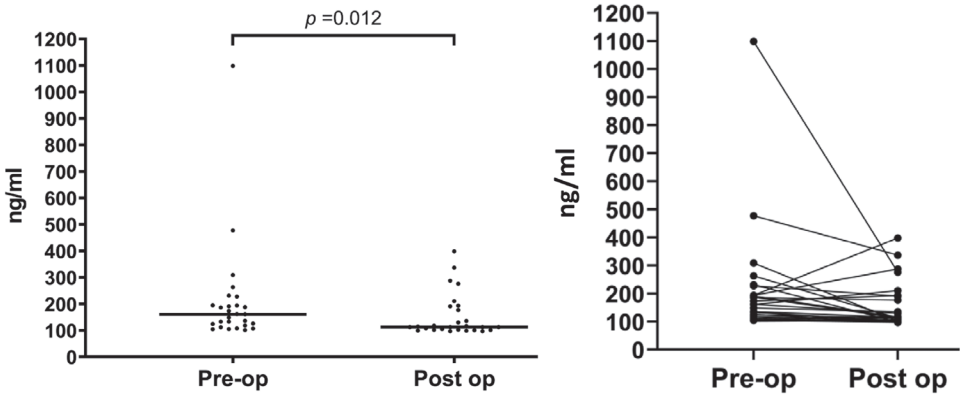

Fig. 3 Changes in serum isoAAT levels observed in patients with OCCC before (preoperative) and after (postoperative) debulking surgery. A, Statistical comparison of isoAAT levels before and after surgery (Wilcoxon sum-ranked test). B, Before-after plots showing the individual changes in serum isoAAT levels from the presurgical to the postsurgical period. isoAAT = alpha 1-antitrypsin isoforms; OCCC = ovarian clear cell carcinoma.

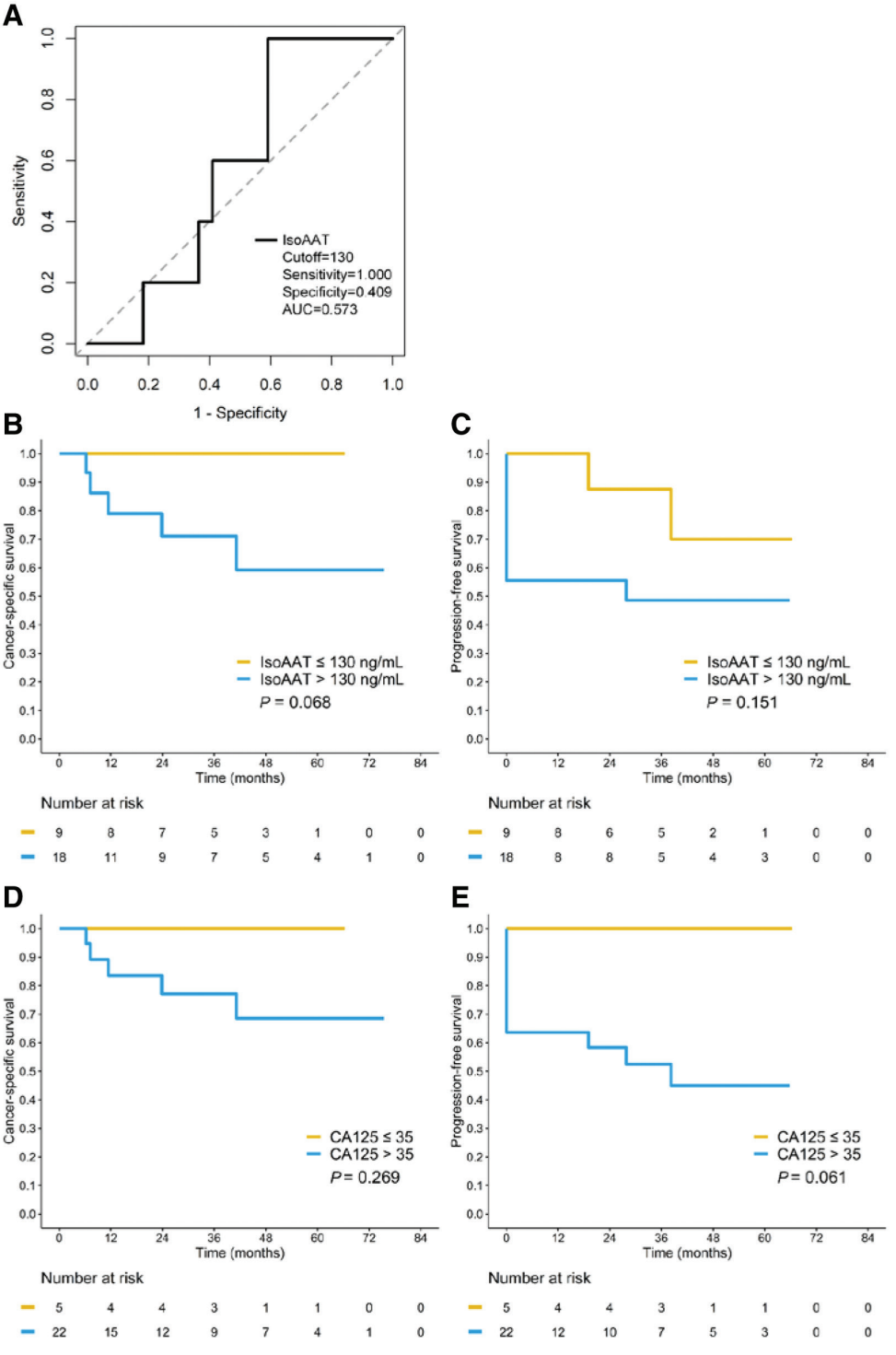

Fig. 4 Kaplan–Meier plots of patients with ovarian clear cell carcinoma (n = 27) in relation to serum isoAAT and CA125 levels. A, The optimal cutoff value for isoAAT (130 ng/mL) in distinguishing patients with OCCC patients who survived versus those who died of disease was identified using receiver operating characteristic curve analysis. B, CSS, and (C) DFS in patients with serum isoAAT levels > 130 ng/mL tended to be less favorable than in those with isoAAT levels ≤ 130 ng/mL (log-rank test, p = 0.068); however, no association between isoAAT levels and DFS was evident. No significant association between CA125 levels and CSS was observed in our cohort (D). However, PFS was marginally less favorable in patients with CA125 > 35 U/mL than in those with CA125 ≤ 35 U/mL (E). CA125 = cancer antigen 125; CSS = cancer-specific survival; DFS = disease-free survival; isoAAT = alpha 1-antitrypsin isoforms; OCCC = ovarian clear cell carcinoma

observed in several tumors, including nonsmall cell lung cancer as well as prostate, breast, colorectal, ovarian, and cervical malignancies.[15] This may occur as a consequence of ectopic production of the molecule by malignant cells or be directly induced by inflammatory mechanisms within the tumor microenvironment. Previous studies have measured circulating AAT levels in patients with ovarian malignancies. For example, Thompson et al[16] have shown that increased levels of AAT

predict unresponsiveness to chemotherapy in ovarian cancer, whereas Yip et al.[17] described an accurate biomarker panel for detecting ovarian malignancies which included both AAT and CA125. Starting from the observation that isoAAT levels are increased in the peritoneal fluid of patients with endometriosis[12,13]; herein, we examined the potential clinical significance of serum isoAAT concentrations in OCCC, an endometriosis-associated malignancy. Collectively, the results of our study suggest that increased isoAAT levels have diagnostic value for differentiating OCCC from benign ovarian tumors. From a prognostic standpoint, OCCC with high isoAAT was associated with a less favorable CSS.

Differently from AAT (reference range: 0.9–2.3 g/L),[18] isoAAT is a low-abundance protein in human serum (median levels in patients with benign ovarian tumor: 125.2 ng/mL). Notably, isoAAT is thought to derive from AAT through different posttranslational modifications (PTMs). While PTMs are known to exert a critical influence on protein function which can play a pathogenetic role in different diseases,[19] it is not possible to conclude whether isoAAT was necessary for sustaining the malignant transformation of endometriosis and/or OCCC tumorigenesis. Future research can examine this hypothesis more rigorously.

Several caveats of our study need to be considered. First, the research had a retrospective design and is thus prone to unavoidable confounding or residual confounding on unmeasured variables. Second, the sample size may not have been sufficient to identify intergroup differences (in terms of study power) and, for that reason, larger cohorts are needed. This also poses a limitation regarding the ability to generalize our conclusions, and replication in independent samples is paramount for ensuring external validity. In addition, the associations between serum isoAAT levels and the clinical characteristics of patients with OCCC (eg, stage) might have been underestimated because of the limited statistical power. Finally, while this study served to evaluate whether serum isoAAT levels decrease after surgery in patients with OCCC, we did not specifically investigate how this biomarker may change in response to chemotherapy or neoadjuvant chemotherapy.

In conclusion, our hypothesis-generating study provides preliminary evidence that patients with OCCC are characterized by increased serum isoAAT levels, whereas concentrations of this molecule were found to be significantly lower in patients with endometriosis or benign ovarian tumors. In addition, serum levels of isoAAT >130 ng/mL were associated with less favorable CSS in patients with OCCC. Taken together, our pilot data indicate that circulating isoAAT levels may be diagnostically useful to distinguish OCCC from benign ovarian tumors and could also serve as a potential prognostic marker. Our findings can prompt larger investigations into the role played by serum isoAAT concentrations as a biomarker in endometriosis-associated OCCC.

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