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Roles of human epididymis protein 4, carbohydrate antigen 125, inhibin B and anti-Müllerian hormone in the differential diagnosis and follow-up of ovarian granulosa cell tumors

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HIGHLIGHTS

- As HE4 and CA125 levels are usually normal in AGCTs, these cannot be used to exclude malignant ovarian tumors.
- Inhibin B is the most accurate single marker for the diagnosis and follow-up of AGCTs.
- Adding AMH to inhibin B may be useful to differentiate AGCTs from ENDOs in premenopausal patients.

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ABSTRACT

Objective. Evaluation of circulating tumor markers in ovarian cancer is crucial for optimal patient care. The goal of this study was to verify the most accurate circulating tumor markers for the diagnosis and follow-up of adult-type granulosa cell tumors (AGCTs).

Methods. The levels of circulating human epididymis protein 4 (HE4) and carbohydrate antigen 125 (CA125), together with AGCT markers inhibin B and anti-Müllerian hormone (AMH), were measured in 135 samples from AGCT patients, 37 epithelial ovarian carcinoma (EOC) patients, and 40 endometrioma (ENDO) patients. The levels were plotted with receiver operating characteristic (ROC) graphs, and the area under the curves (AUC) of the different markers were calculated and compared.

Results. HE4 levels were significantly lower in AGCTs than in EOCs ($p < 0.0001$). CA125 levels were above 35 IU/l in 25% of AGCT patients and 47.5% of ENDO patients, whereas inhibin B and AMH levels were elevated only in patients with AGCTs. In the AUC comparison analyses, inhibin B alone was sufficient to differentiate AGCT from EOC. In differentiating AGCT from ENDO, inhibin B and AMH performed similarly, and the combination of inhibin B and AMH increased the accuracy compared to either marker alone (sensitivity, 100%; specificity, 93%). Among AGCT patients, inhibin B was the best marker for detecting the presence of AGCT.

Conclusions. HE4 and CA125 levels were low in AGCTs, and inhibin B was the most accurate circulating biomarker in distinguishing AGCTs from EOCs and from ENDOs. Inhibin B was also the best single marker for AGCT follow-up.

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1. Introduction

Differential diagnostics of an ovarian tumor is a common, albeit demanding clinical challenge, and critical for optimal patient care [1,2].

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Typically, a combination of symptoms, clinical status, sonographic features, and laboratory analyses are involved in evoking the suspicion of a potentially malignant ovarian tumor. Circulating tumor markers, such as carbohydrate antigen 125 (CA125), are commonly utilized to evaluate the risk of malignancy of the most common ovarian tumors of epithelial origin [3,4]. In the differential diagnosis of pelvic masses, human epididymis protein 4 (HE4) is considered to be superior to CA125 because the levels of HE4 are less influenced by the presence of endometriomas and other benign ovarian tumors [5–7]. Furthermore, HE4 shows better diagnostic sensitivity in the early stages of epithelial ovarian carcinomas (EOCs) [8,9]. However, nontumor-related factors, such as smoking, aging, and renal impairment, specific to individual patients, may also lead to increased HE4 levels [10,11] Specific algorithms have been developed to enhance the diagnostic potential of single serum markers. These include the Risk of Malignancy Index, which combines sonographic findings, menopausal status, and serum CA125 levels [12], and the Risk of Ovarian Malignancy Index, which combines CA125 and HE4 levels with the menopausal status in a mathematical algorithm [13]. Although several studies have compared the diagnostic performance of these algorithms, no clear conclusions have been drawn regarding their superiority [8,14,15].

After EOCs, adult-type ovarian granulosa cell tumors (AGCTs) are the second-most common ovarian malignancy, representing 5% of all ovarian cancers. AGCTs are generally detected at an early stage and are treatable with curative surgery [16–18]. The prognosis of AGCTs is usually excellent, with 97–98% 5-year survival [17,18]. However, every third patient, even those with early-stage AGCTs, relapses, and half of those patients die due to the disease [17,18]. Although the histological diagnosis of AGCT can be difficult, a unique somatic mutation in *FOXL2* has been shown to assist in unequivocal diagnosis [19,20]. Inhibin B and anti-Müllerian hormone (AMH, also known as Müllerian-inhibiting substance) have been established as follow-up markers for AGCTs [21, 22]. Among EOC markers data concerning AGCTs are limited. According to two reports, CA125 has been reported to be elevated in a subset of AGCTs [23,24]. HE4 is a relatively novel serum marker for ovarian cancer, and its levels in AGCTs are unknown.

Thus far only few studies have been focusing on the preoperative evaluation and differential diagnosis AGCTs from other ovarian tumors [23]. Further, the available differential diagnostic algorithms (e.g., the Risk of Ovarian Malignancy Index) have been developed solely for epithelial carcinomas. National Cancer Center Network (NCCN) guidelines suggest that inhibin could be measured when less common ovarian malignancies are suspected [4]. However, the evidence behind the guidelines on AGCTs is sparse, and there is a need for a comparative analysis of circulating tumor markers that can be utilized in differential diagnostics of AGCTs. Also, after the suspicion of an AGCT and during the post-treatment follow-up, there is inconsistent evidence on the preferred tumor marker [25]. Utilizing a large prospective cohort of AGCT patients and controls, this study aimed to evaluate the levels of circulating tumor markers HE4, CA125, inhibin B, and AMH and their ability to differentiate AGCTs from other ovarian tumors at diagnosis. An additional aim was to determine their potential roles as circulating biomarkers, as well as validate a novel detection method for AMH, in the follow-up of AGCT patients.

2. Materials and methods

2.1. Patients and serum samples

Altogether 135 serial blood samples were obtained from 82 AGCT patients treated at the Department of Obstetrics and Gynecology, Helsinki University Central Hospital. From 2007 onwards we have collected blood samples from consenting patients with verified AGCT, and all the available samples drawn during 2007–2011 were included into the study. All AGCTs were tested positive for the *FOXL2* (c.402C>G; C134W) mutation. Clinical data were collected from the patients' medical records, including data on smoking and blood creatinine levels, which are known to affect the interpretation of data on some of the studied tumor markers. The patients were diagnosed between 1962 and 2009, and staging of the primary tumors was done according to the FIGO 2009 criteria. The median size of the AGCTs was 7.0 cm (range 2.0–40.0 cm). The median follow-up time of the AGCT patients after blood sampling was 4.7 years (2.3–6.4 years). The ethics committee of Helsinki University Central Hospital and the National Supervisory Authority for Welfare and Health in Finland approved the study, and all the patients gave their informed consent.

The preoperative and prechemotherapy samples were obtained from the AGCT patients within a month before surgery or chemotherapy, and the follow-up samples were obtained during routine clinical follow-up visits, after a minimum of three months from tumor treatment. The AGCT samples were dichotomized according to the presence of macroscopic disease (with disease [WD] or disease-free [DF]) as evidenced at the operation or by imaging with ultrasound or computer tomography and a clinical examination. Clinical follow-up visits included a gynecologic examination and ultrasonography and other imaging when indicated. Inhibin B, but no other marker levels, were available for the clinician during the follow-up of the majority of the AGCT patients. The AGCT WD group consisted of 28 preoperative samples, five samples drawn at the onset of chemotherapy and three samples drawn during the follow-up. In the AGCT DF group all the samples were drawn during the follow-up. All the samples were also classified as follows according to the patient's menopausal status at sample retrieval: "premenopausal" if the patient had one or two ovaries and menopause was not indicated in the medical records (e.g., cessation of regular bleeding, presence of menopausal symptoms, use of hormonal replacement therapy) and "postmenopausal" if both ovaries had been removed, independent of age, or the patient was postmenopausal according to her medical history. The sample details are summarized in Table 1.

To evaluate the role of circulating tumor markers in differential diagnostics of AGCTs, marker levels in 36 AGCT (WD) samples were compared to those in preoperative samples derived from 37 patients with EOCs and 40 patients with ENDOs (Table 1). The samples were drawn within one month before the surgery. The EOC tumors were classified histologically as high-grade serous ($n = 31$, 84%), mucinous ($n = 2$, 5%), endometrioid ($n = 3$, 8%), or clear cell types ($n = 1$, 3%). The ENDO group consisted of women undergoing surgery due to an ovarian endometrioma. The ENDO group was significantly different from the

Table 1
Sample details (n).

	AGCT			
	WD	DF	EOC	ENDO
	36	99	37	40
Year of sample retrieval	2007–2011	2007–2011	2009–2014	2005–2007
Age of the patient at sample retrieval, years[a]	60 (36–80)	59 (25–86)	61 (29–79)	32 (26–47)
Primary[b]	17	n/a	37	40
Stage I	17	n/a	5	0
Stage II	0	n/a	3	0
Stage III	0	n/a	16	15
Stage IV	0	n/a	16	24
Recurrent[c]	19	n/a	0	0
PreMP[c,d]	6	12	7	40
PostMP[c,d]	30	87	30	0

Abbreviations: WD; with disease, DF; disease-free, n/a; not applicable, MP; menopausal.
[a] Median (range).
[b] Staging according to FIGO 2009 in AGCTs and EOCs, and according to American Society for Reproductive Medicine in ENDOs. Stage is available only for primary tumors in WD-group, as in the surgery of recurrent tumors staging procedure is not performed.
[c] Serial samples from multiple recurrences.
[d] MP status at sample retrieval; "premenopausal" (PreMP) if the patient had and one or two ovaries, and menopause was not indicated in the medical records, and "postmenopausal" (PostMP) if the both ovaries had been removed, or the patient was postmenopausal according to medical history.

AGCT and EOC groups, as it consisted of young, premenopausal patients, whereas the age distribution and menopause status of the AGCT and EOC groups were similar ($p > 0.05$). In order to reveal the optimal follow-up marker, the tumor marker levels in the 36 AGCT WD samples were compared to the 99 AGCT DF samples. No post-treatment samples were available from EOC and ENDO patients.

Serum and plasma samples were prepared and stored at −80 °C until the analysis. Serum HE4 (pM) was analyzed using an enzyme-linked immunosorbent assay (ELISA) (Fujirebio Diagnostics Inc., Malvern, PA, U.S.), according to the manufacturer's instructions. Serum CA125 (IU/ml) levels were analyzed using a chemiluminescent microparticle immunoassay (Architect CA 125 II) on an Abbott Architect i2000 system (Abbott diagnostics, Abbott Park IL, U.S.), and serum inhibin B (ng/l) levels were analyzed using an Inhibin B Gen II ELISA (A81303), with inhibin B Gen II calibrators and controls (A81304) (Beckman Coulter, CA, U.S.). Creatinine (μmol/l) levels were analyzed using an enzymatic assay (Roche Modular 8000 clinical chemistry analyzer; Roche Diagnostics, Penzberg, Germany) in the Helsinki University Hospital laboratory. The glomerular filtration rate (GFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula [26]. AMH (ng/ml) levels were analyzed from plasma samples with an ultrasensitive AMH ELISA (AL-105i) from AnshLabs (AnshLabs, Webster, TX, U.S.).

The analyses were performed using both continuous values and cut-off levels. The cut-off level for HE4 was set on 150 pM according to the manufacturer's instructions. Patients were regarded as having impaired renal function if the GFR was <60 ml/min/1.73 m^2 [27]. The cut-off level for CA125 was 35 IU/l. For inhibin B and AMH, the cut-off levels were determined according to the manufacturers' instructions. For premenopausal patients, the cut-off limits for inhibin B and AMH was <200 ng/l and <13 μg/l respectively. For postmenopausal patients, the cut-off limits were <16 ng/l and <0.2 μg/l, respectively.

3. Statistical analyses

The levels of the markers did not follow a normal distribution, and the between-group comparisons were therefore analyzed using the Mann–Whitney *U* test or Spearman's Rho. Receiver operating characteristic (ROC) curves were constructed, and the area under the curve (AUC) values were calculated, together with their 95% confidence intervals. All the study samples were included in the ROC analyses. Due to the rarity of the disease, multiple circulating tumor marker measurements (both pre-treatment (WD) and follow-up (WD or DF) samples) of the AGCT patients were included to increase the precision of the estimates of sensitivity and specificity. As the repeated measurements-ROC curves gave falsely optimistic estimates, the measurements were utilized as independent data. Thus, the ROC curves should be viewed as descriptive only. For the ROC-curve calculations, observations below the detection limit were replaced with DL/2 values. Correlated ROC curves were compared nonparametrically [28]. The associations between continuous variables and disease status (AGCT WD, EOC, ENDO, AGCT DF) were studied using a mixed model repeated measures analysis if all the values were above the detection limit and with a mixed effects Tobit model [29] if some variables were below the detection limit. For the ROC analysis using the cut-off data the samples were dicotomized as either high or normal based on the aforementioned cut-off-levels. Statistical analyses were done using JMP Pro, version 11.0 and SAS, version 9.3 (SAS Inc., Cary, NC, U.S.).

4. Results

4.1. HE4 and CA125 levels were lower in AGCT patients than in EOC patients: high levels indicated a malignant tumor of epithelial origin

HE4 levels were significantly elevated in the EOC group when compared to those of the other groups ($p < 0.0001$ in all pairwise comparisons). Furthermore, HE4 levels were increased in both AGCT WD and DF patients when compared to ENDO patients due to the younger mean age of the patients in the ENDO group. Interestingly, HE4 levels were also higher in AGCT WD patients when compared to AGCT DF patients ($p = 0.034$). HE4 levels were correlated positively with serum creatinine levels (Supplemental Fig. S1). After excluding patients with renal insufficiency (estimated GFR ≤ 60 ml/min/1.73 m^2), the results revealed no statistical difference in HE4 levels between the AGCT WD and DF patients, whereas the differences between the other groups were sustained (data not shown).

HE4 levels were above the reference limit (150 pM) in only four AGCT patients (Supplemental Table S1). Apart from one (a young patient; aged 25 years), all of these patients had impaired renal function based on a decreased GFR. In the AGCT WD group only one patient had high HE4 level reaching 256.1 pM, which could be explained by a chronic glomerulonephritis and renal impairment. Elevated HE4 levels were measured also in her two DF samples (197.4 pM and 375.6 pM). The serum HE4 levels of the AGCT patients did not correlate with tumor size or tumor stage (data not shown).

In common with HE4 levels, serum CA125 levels were significantly higher in patients with EOCs compared to AGCTs (Fig. 1B). The CA125 levels were also significantly increased in AGCT WD patients compared to DF patients ($p = 0.0001$). However, the elevation in CA125 levels of the AGCT WD patients was very modest, with only 25% of the levels exceeding the limit of 35 IU/l. The levels of the AGCT WD and ENDO patients were similar. The levels of CA125 were elevated in three samples (CA125 37–71 IU/l) obtained from one AGCT patient (ID 159), probably due to simultaneous endometriosis. Interestingly, one

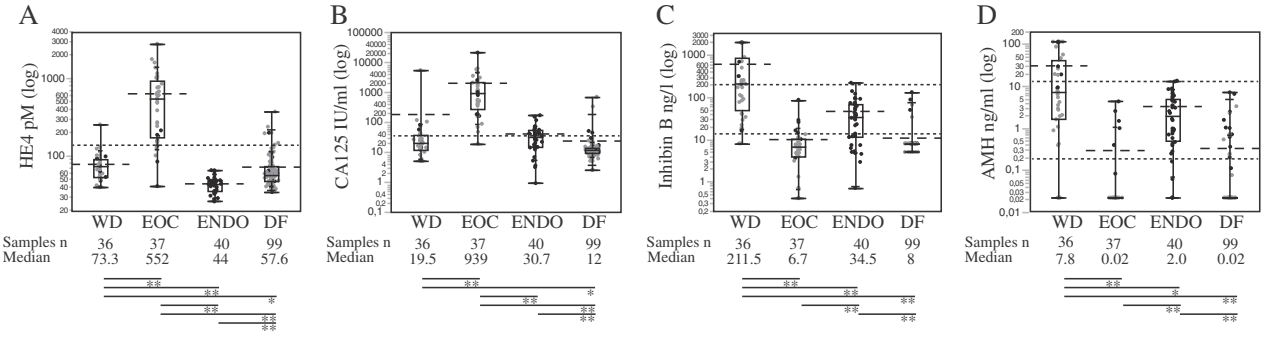

Fig. 1. The concentrations of HE4 (A), CA125 (B), inhibin B (C), and AMH (D) levels in AGCT WD, EOC, ENDO, and AGCT DF patients. The data are shown as boxplots, and the dots represent measurements of individual patients. The black dots represent premenopausal patients, and the gray dots denote postmenopausal patients. Cut-off levels for each marker are depicted as dashed lines. The levels of the marker are represented on a logarithmic scale. In comparisons between the groups, the asterisks indicate significant statistical difference. *$p < 0.05$, **$p < 0.0001$. Abbreviations: AGCT, adult-type granulosa cell tumor; WD, with disease; DF, disease free; EOC, epithelial ovarian carcinoma; ENDO, endometrioma.

patient (ID 122) had extremely high CA125 levels: 5400 IU/ml (WD) and 696 IU/ml (DF). No specific explanations for these high levels were found, and the differences between groups remained the same when this patient was excluded as an outlier from the analysis. CA125 levels did not correlate with tumor size or stage in the AGCT patients (data not shown).

4.2. Inhibin B and AMH levels were increased in patients with AGCTs

Utilizing this prospective series, roles of inhibin B and AMH were analyzed in the diagnostic setting of AGCTs. Inhibin B and AMH levels were significantly higher in AGCT WD patients when compared to those of EOC and ENDO patients (Fig. 1). Inhibin B and AMH levels were also higher in ENDO patients when compared to those of the AGCT DF and EOC groups. These findings are related to the characteristics of the ENDO group (i.e., mainly younger patients, with functional ovaries). Among the ENDO patients, 1/40 (2.5%) had elevated inhibin B levels, and 2/40 (5%) had elevated AMH levels above the normal range. Inhibin B and AMH levels were also higher in AGCT WD samples when compared to DF samples, confirming their roles as markers in the follow-up of AGCT patients.

4.3. Inhibin B was the most accurate single preoperative marker to differentiate AGCTs from EOCs and ENDOs

To evaluate the performance of the individual markers and combinations of markers in the differential diagnostics of AGCT, receiver operating characteristic (ROC) curve analyses were performed, first using the continuous value data. In differentiating AGCTs from EOCs, all single markers were highly accurate, with AUCs between 0.92 and 0.97, and combinations of the markers did not improve their accuracy (Fig. 2A). In distinguishing AGCTs from ENDOs, the accuracies of single markers were lower, and combining the markers seemed to improve their performance (Fig. 2B). When evaluating the markers in the follow-up of AGCT patients, the accuracies of inhibin B and AMH were higher than those of the other single markers (Fig. 2C).

To validate these findings, ROC-AUC analyses were performed, using dichotomized values by cut-off levels for each marker. In differentiating AGCTs from EOCs, all the markers performed well, with AUC values between 0.86 for CA125 and 0.96 for inhibin B (Supplemental Fig. S2). In discriminating AGCTs from ENDOs, the utilization of the cut-off levels improved the accuracy of the markers, especially that of inhibin B and AMH. Combining inhibin B with any of the markers resulted in AUC values close to or 1.0, indicating high accuracy in all the patient groups.

In ROC-AUC comparison analyses using the dichotomized data, inhibin B was the most accurate single marker in the primary diagnosis of AGCTs, and there was no benefit in adding other markers to inhibin B when differentiating between AGCTs and EOCs (Table 2). Similar results were obtained in ROC-AUC comparison analyses using continuous value data (data not shown). Of the epithelial markers, HE4 and CA125 were equally accurate in distinguishing between AGCT and EOCs. In differentiating between AGCTs and ENDOs, inhibin B and AMH performed equally well. However, adding AMH to inhibin B significantly increased the accuracy compared to inhibin B ($p = 0.036$) or AMH ($p = 0.004$) alone, resulting in sensitivity of 100% and specificity of 93% (Table 3). During AGCT follow-up, inhibin B performed as well as AMH as a single marker (Table 2). Inhibin B and AMH together were superior to AMH alone ($p = 0.001$) but not inhibin B alone ($p > 0.05$).

5. Discussion

AGCTs constitute a unique entity of ovarian-sex cord stromal-derived neoplasms, originating from the granulosa cells of the ovary. Based on the present study, inhibin B is the preferred marker for differential diagnostics and follow-up of AGCTs. Further, the levels of epithelial ovarian cancer markers HE4 and CA125 are usually below normal reference limits in AGCT patients. As the knowledge on the origins of ovarian cancer has increased [30,31], we nowadays appreciate that the different ovarian cancer subtypes have divergent diagnostic paths and expression levels of circulating tumor markers [32,33]. The differential diagnostics delineates the referral of patients with suspected ovarian cancer to specialized centers, which is associated with significantly improved survival, underlining the value of accurate preoperative diagnostics [1,2]. Although relatively rare, ovarian AGCTs are malignant, with a tendency toward late recurrence in up to 30% of cases, leading to increased mortality [17,18]. Surgery and complete tumor removal are the cornerstones in the treatment of both primary and relapsed disease and determine the survival of AGCT patients [34]. Spontaneous or

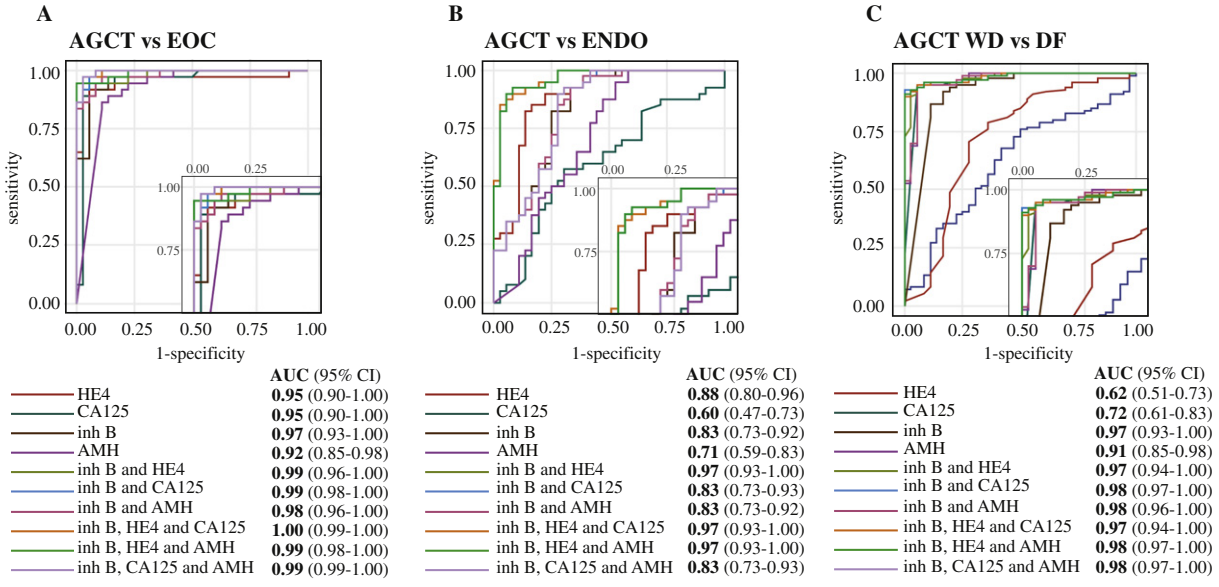

Fig. 2. The ROC-AUC analyses for HE4, CA125, inhibin B, AMH, and marker combinations using continuous values. ROC analyses depicting the accuracy of single markers and combinations of markers in distinguishing between AGCT WD and EOC (A), AGCT WD and ENDO (B), and AGCT WD and DF samples (C). The inset in the right lower corner represents an enlargement of the upper left corner of the ROC graph. Abbreviations: ROC-AUC, receiver operator characteristic-area under the curve; AGCT, adult-type granulosa cell tumor; WD, with disease; DF, disease free; EOC, epithelial ovarian carcinoma; ENDO, endometrioma.

Table 2
p-Values of the AUC comparisons using cut off values.

	AGCT vs EOC				AGCT vs ENDO				AGCT WD vs DF			
Marker	HE4[b]	CA125[b]	Inh B[b]	AMH[b]	HE4[b]	CA125[b]	Inh B[b]	AMH[b]	HE4[b]	CA125[b]	Inh B[b]	AMH[b]
HE4[a]		ns	ns	ns		ns	<0.0001[b]	<0.0001[b]		0.021[b]	<0.0001[b]	<0.0001[b]
CA125[a]	ns		0.046[b]	ns	ns		<0.0001[b]	<0.0001[b]	0.021[a]		<0.0001[b]	<0.0001[b]
Inh B[a]	ns	0.046[a]		ns	<0.0001[a]	<0.0001[a]		ns	<0.0001[a]	<0.0001[a]		ns
AMH[a]	ns	ns	ns		<0.0001[a]	<0.0001[a]	ns		<0.0001[a]	<0.0001[a]	ns	
Inh B HE4[a]	0.005[a]	0.001[a]	ns	0.018[a]	<0.0001[a]	<0.0001[a]	ns	ns	<0.0001[a]	<0.0001[a]	ns	ns
Inh B CA125[a]	0.006[a]	0.0004[a]	ns	0.009[a]	<0.0001[a]	<0.0001[a]	0.041[a]	0.044[a]	<0.0001[a]	<0.0001[a]	ns	ns
Inh B AMH[a]	0.005[a]	0.0004[a]	ns	0.008[a]	<0.0001[a]	<0.0001[a]	0.036[a]	0.004[a]	<0.0001[a]	<0.0001[a]	ns	0.001[a]
Inh B HE4 CA125[a]	0.005[a]	0.0004[a]	ns	0.008[a]	<0.0001[a]	<0.0001[a]	0.041[a]	0.045[a]	<0.0001[a]	<0.0001[a]	ns	ns
Inh B HE4 AMH[a]	0.005[a]	0.0004[a]	ns	0.008[a]	<0.0001[a]	<0.0001[a]	0.036[a]	0.004[a]	<0.0001[a]	<0.0001[a]	ns	0.001[a]
Inh B CA125 AMH[a]	0.005[a]	0.0004[a]	ns	0.008[a]	<0.0001[a]	<0.0001[a]	0.045[a]	0.003[a]	<0.0001[a]	<0.0001[a]	ns	0.0008[a]

The index in the *p*-value indicates the significantly superior marker.

iatrogenic tumor rupture at surgery, converting a typical Stage Ia tumor to Stage Ic tumor, results in a significantly increased risk of relapse [18,35]. This further emphasizes the importance of pretreatment evaluations in ensuring that patients with suspected AGCTs are referred to specialized oncology units.

To improve the differential diagnosis and treatment of patients with AGCTs, the present study evaluated a panel of serum markers in an established AGCT patient cohort [36]. Although the cohort size of this study may be limited, the circulating biomarkers studied herein have not been examined in any larger, molecularly verified AGCT cohorts. Interestingly, recent evidence pointed to the utility of a 402C>G mutation in the *FOXL2* gene in circulating tumor DNA in the diagnosis of AGCTs in 30% of patients [37]. Although this diagnostic method is highly specific, its clinical utilization is compromised by a laborious assay and low sensitivity. Thus, circulating biomarkers are needed in the clinical preoperative diagnostics of ovarian tumors.

Consistent with the lack of HE4 protein expression in AGCT tissue [32] serum HE4 levels were elevated in only a minority of AGCT patients in the present study. Serum HE4 levels were positively associated with creatinine levels, consistent with previous suggestions that renal insufficiency, smoking, and aging can all increase the levels of HE4 [10,11]. In the present AGCT cohort, a significant proportion of high HE4 levels could be explained by impaired renal function, underlining the importance of simultaneous creatinine measurements when evaluating HE4 levels, especially in older patients. HE4 levels are not elevated in endometriomas or other benign tumors [5,6], and the HE4 marker is considered more specific than CA125 in EOCs [6]. Moreover, HE4 fluctuates very little during the menstrual cycle and is unaffected by commonly used hormonal medications [38]. Clinically, the findings of the present study are consistent with those in the literature [5,6], with high HE4 levels clearly indicating a malignant epithelial tumor.

It is important to keep in mind that normal HE4 levels do not rule out ovarian malignancy. In the present study, in common with the findings for HE4, high CA125 levels were indicative of EOCs, and CA125 levels were mostly below normal reference limits in AGCTs. The slight elevation in CA125 levels in a subset of AGCTs (both WD and DF), as well as in ENDO patients, alludes to the diminished specificity of CA125 as a marker for epithelial ovarian tumors.

Although several reports have demonstrated the clinical utility of inhibin B and AMH in AGCT surveillance [25], the evidence on their role in discriminating AGCTs from other ovarian tumors has been lacking. The present study indicated that inhibin B was the most accurate marker in differentiating AGCTs from EOCs and ENDOs. Regarding different ovarian cancer subtypes, previous reports have shown that inhibin B levels can be elevated in mucinous epithelial carcinomas [25]. In the present study, two patients with mucinous carcinomas exhibited elevated levels of both HE4 and CA125 and normal levels of inhibin B (5.2 and 3.6 ng/l). A larger cohort of mucinous carcinomas is needed to thoroughly evaluate the ability of inhibin B to differentiate AGCTs from mucinous EOCs.

According to our previous report using different methodology and a larger, mostly historical cohort [21], inhibin B and AMH measurements performed equally in AGCT follow-up and their combination was superior to inhibin B alone. However, problems with AMH assays have precluded the clinical usability of the marker [39]. In the present study, using a novel AMH assay [40], AMH performed as well as inhibin B in the AGCT follow-up, but there was no significant benefit in combining them. Nevertheless, there were fewer samples and a shorter follow-up in the present when compared to the earlier study. We noted that AMH measurements increased the accuracy of the differential diagnostics of AGCTs in patients with ENDOs. Thus, measuring AMH levels, in addition to inhibin B levels, might be useful in the differential diagnostics of AGCTs from ENDOs in premenopausal patients. However, as the sensitivity and specificity of inhibin B alone are already high, the clinical relevance of combining it with AMH may benefit only a limited subset of patients.

We conclude that inhibin B is the most accurate marker for the preoperative diagnosis of AGCTs, as it is able to differentiate AGCTs from

Table 3
Sensitivities and specificities of markers and marker combinations.

	AGCT vs EOC		AGCT vs ENDO		AGCT WD vs DF	
	$n = 73$		$n = 76$		$n = 135$	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
HE4	97	84	3	100	97	5
Ca125	75	97	75	48	25	96
Inhibin B	92	100	92	98	92	98
AMH	100	83	83	95	83	92
InhB and HE4	92	100	92	98	92	98
InhB and CA125	100	97	92	98	92	98
InhB and AMH	100	100	100	93	100	92
InhB, HE4 and CA125	100	100	92	98	92	98
InhB, HE4 and AMH	100	100	100	93	100	92
InhB, CA125, and AMH	100	100	100	95	100	92

both EOCs and ENDOs. To aid the preoperative diagnosis of a potential AGCT, we recommend that inhibin B and AMH levels should be measured in premenopausal women and that inhibin B levels should be measured in postmenopausal women. HE4 and CA125 levels are generally low in AGCTs, and the patient's age, as well as creatinine levels and GFRs, should be considered in the interpretation of HE4 levels. It is important to emphasize that a malignant ovarian tumor cannot be excluded based on normal levels of HE4 and/or CA125. Thus, the measurement of circulating biomarkers has a crucial role in ensuring optimal treatment, including referral to a specialized oncology unit, for patients with a clinical suspicion of ovarian cancer.

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Conflict of interest statement

The authors declare no conflicts of interest.

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