Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

Cancer Epidemiology, Biomarkers & Prevention

Research Article

A Novel Approach to Predict the Likelihood of Specific Ovarian Tumor Pathology Based on Serum CA-125: A Multicenter Observational Study

Ben Van Calster[1,2], Lil Valentin[4], Caroline Van Holsbeke[1,5], Jing Zhang[6], Davor Jurkovic[7], Andrea Alberto Lissoni[9], Antonia Carla Testa[10], Artur Czekierdowski[11], Daniela Fischerová[12], Ekaterini Domali[13], Gregg Van de Putte[5], Ignace Vergote[1], Sabine Van Huffel[2,3], Tom Bourne[1,8], and Dirk Timmerman[1]

Abstract

Background: The CA-125 tumor marker has limitations when used to distinguish between benign and malignant ovarian masses. We therefore establish likelihood curves of six subgroups of ovarian pathology based on CA-125 and menopausal status.

Methods: This cross-sectional study conducted by the International Ovarian Tumor Analysis group involved 3,511 patients presenting with a persistent adnexal mass that underwent surgical intervention. CA-125 distributions for six tumor subgroups (endometriomas and abscesses, other benign tumors, borderline tumors, stage I invasive cancers, stage II–IV invasive cancers, and metastatic tumors) were estimated using kernel density estimation with stratification for menopausal status. Likelihood curves for the tumor subgroups were derived from the distributions.

Results: Endometriomas and abscesses were the only benign pathologies with median CA-125 levels above 20 U/mL (43 and 45, respectively). Borderline and invasive stage I tumors had relatively low median CA-125 levels (29 and 81 U/mL, respectively). The CA-125 distributions of stage II–IV invasive cancers and benign tumors other than endometriomas or abscesses were well separated; the distributions of the other subgroups overlapped substantially. This held for premenopausal and postmenopausal patients. Likelihood curves and reference tables comprehensibly show how subgroup likelihoods change with CA-125 and menopausal status.

Conclusions and Impact: Our results confirm the limited clinical value of CA-125 for preoperative discrimination between benign and malignant ovarian pathology. We have shown that CA-125 may be used in a different way. By using likelihood reference tables, we believe clinicians will be better able to interpret preoperative serum CA-125 results in patients with adnexal masses. *Cancer Epidemiol Biomarkers Prev; 20(11); 2420–8. ©2011 AACR.*

Introduction

An accurate diagnosis of adnexal masses prior to surgery is important to optimize the prognosis of women with ovarian cancer. A correct diagnosis leads to more appropriate management and improved referral patterns. This is very important, as research has clearly shown the effect of type of surgery and surgeon on prognosis (1–3). Moreover, the clinical management of patients with suspected ovarian cancer improves with diagnostic accuracy (4).

The cancer antigen 125 (CA-125) is a glycoprotein which is encoded in the *MUC16* mucin gene (5). It is being assessed in ovarian cancer screening trials as well

Authors' Affiliations: [1]Department of Obstetrics and Gynecology, University Hospitals K.U. Leuven; [2]Department of Electrical Engineering, [3]IBBT-K.U. Leuven Future Health Department, Katholieke Universiteit Leuven, Leuven, Belgium; [4]Department of Obstetrics and Gynecology, Skåne University Hospital Malmö, Lund University, Sweden; [5]Department of Obstetrics and Gynecology, Ziekenhuis Oost-Limburg, Genk, Belgium; [6]Department of Interventional Ultrasound, Chinese PLA General Hospital, Beijing, China; [7]Gynecology Diagnostic and Outpatient Treatment Unit, University College Hospital, [8]Department of Obstetrics and Gynecology, Imperial College, Hammersmith Campus, London, United Kingdom; [9]Clinica Ostetrica e Ginecologica, Ospedale San Gerardo, Università di Milano-Bicocca, Monza; [10]Istituto di Clinica Ostetrica e Ginecologica, Università Cattolica del Sacro Cuore, Rome, Italy; [11]1st Department of Gynecology, Medical University of Lublin, Lublin, Poland; [12]Oncogynecological Center, Department of Obstetrics and Gynecology, First Faculty of Medicine and General University Hospital, Charles University, Prague, Czech Republic; and [13]Department of Obstetrics and Gynecological Ultrasound, Alexandra Maternity Hospital, Athens University, Athens, Greece

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Ben Van Calster, Department of Electrical Engineering, K.U. Leuven, Kasteelpark Arenberg 10 bus 2446, B-3001 Leuven, Belgium. Phone: 0032-1632-1925; Fax: 0032-1632-1970; E-mail: ben.vancalster@esat.kuleuven.be

doi: 10.1158/1055-9965.EPI-11-0422

Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

as being used clinically for the characterization of adnexal masses because it is often increased in the presence of ovarian cancer (5–10). In their meta-analysis of 17 studies comprising 2,374 patients who underwent surgery for an ovarian tumor, Medeiros and colleagues obtained a pooled sensitivity with regard to ovarian malignancy (borderline malignancy or invasive cancer) of 80% and a pooled specificity of 75% using a CA-125 value of 35 U/mL or more to indicate malignancy (10). However, it is widely recognized that CA-125 has limitations when used in isolation (11). One problem is that CA-125 levels are affected by a number of conditions such as pelvic inflammatory disease, endometriosis, large uterine fibroids, ovarian fibroma, peritoneal implants, lung disease, liver cirrhosis, tuberculosis, ascites, smoking, obesity, caffeine use, Caucasian ethnicity, and previous hysterectomy (12–16). More importantly, relatively low CA-125 levels are seen commonly in patients with borderline tumors and stage I invasive ovarian cancers (7–9, 13, 17, 18).

The International Ovarian Tumor Analysis (IOTA) study group has previously investigated the role of CA-125 in the preoperative assessment of adnexal masses (9, 19, 20). We observed much higher CA-125 levels in endometriomas, abscesses, and fibromas than in other benign masses, and much lower levels in borderline tumors than in invasive tumors, with only stage II–IV invasive cancers having consistently elevated CA-125 levels (9). We found that the subjective assessment of the ultrasound image of an adnexal mass by an experienced ultrasonographer was superior to serum CA-125 for the diagnosis of malignancy and that adding information on the CA-125 level to subjective evaluation of ultrasound images did not improve diagnostic performance (8, 20, 21). Moreover, CA-125 appeared to be replaceable by other variables in mathematical models developed to predict malignancy in adnexal masses (19). These are important results. However, they were based on only 809 patients selected on the basis of the availability of CA-125 levels from a larger data set of 1,066 patients from 9 European clinical centers.

In this study, we describe CA-125 levels in tumors with different pathology based on an expanded multicenter data set of 3,511 patients using multiple imputation to deal with missing CA-125 levels. The main aim was to estimate distributions of serum CA-125 levels for specific tumor subgroups and to use these distributions to establish the likelihood of a specific tumor subgroup based on the CA-125 value and the menopausal status of the patient.

Materials and Methods

Design and setting

This is a multicenter observational cross-sectional study of patients presenting with at least one persistent adnexal (ovarian, paraovarian, or tubal) mass that later underwent surgery. Data were collected prospectively between 1999 and 2007 within the frame of the IOTA study (22–25). The key aims of the IOTA studies are the development and validation of clinical risk prediction models to diagnose malignancy (22–25). The "gold standard" in these studies to date has been the pathologic examination of removed adnexal tumor tissues at surgery. Accordingly, all masses in the study are lesions that have been selected in each center to require surgical intervention.

The IOTA group has collected data in 3 phases (1, 1b, and 2; Supplementary Table S1). Patients were recruited in 11 oncology referral centers and 3 referral centers for ultrasonography, both based in university hospitals, and in 7 public hospitals in 9 countries. For phase 1, patients were recruited between 1999 and 2002, for phase 1b, between 2002 and 2005, and for phase 2, between 2005 and 2007. Supplementary Table S1 lists the centers involved in each IOTA phase. The research protocols were ratified by the local ethical committee at each recruitment center.

Patients

Patients presenting with at least one persistent adnexal mass and who were examined with ultrasonography by a principal investigator were eligible for inclusion, conditional on oral informed consent prior to the ultrasound scan and surgery. The ethical committees advised that written consent was not needed, as standard clinical care would be provided. If more than one mass was detected, the mass with the most complex ultrasound morphology was used for the measurement of tumor features. When masses with similar morphology were observed, the largest mass or the one most easily accessible by ultrasound was used. Exclusion criteria were pregnancy, refusal of transvaginal ultrasonography, and surgical removal of the mass more than 120 days after the ultrasound examination.

Data collection

For each patient, a standardized history was taken to collect clinical variables of which menopausal status is the principal variable used in this study. A woman was classified as postmenopausal if after the age of 40 years, she reported an absence of menstruation for at least 1 year, unless the amenorrhea was caused by medication, disease, or pregnancy. Patients aged 50 years, who had undergone a hysterectomy such that the time of menopause could not be determined, were considered postmenopausal. Patients younger than 50 years, who had undergone hysterectomy without bilateral oophorectomy, were considered premenopausal. More information on the clinical variables that were recorded can be found in a previous report (22).

All patients underwent a standardized transvaginal ultrasound examination to investigate the tumor, as described in the IOTA consensus report (26). Transabdominal sonography was carried out for large masses that could not be visualized in full by a transvaginal probe.

Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

Information on more than 40 ultrasound morphologic and blood flow variables was collected using grayscale and color Doppler ultrasound imaging.

The reference standard was the pathologic diagnosis of the mass following surgical removal by laparotomy or laparoscopy as judged appropriate by the surgeon. The pathology was divided into 20 categories (11 benign and 9 malignant ones) as shown in Table 1. We also classified the tumors into 1 of 6 tumor subgroups based on *a priori* knowledge and median CA-125 levels: endometriomas and abscesses, benign tumors other than endometriomas and abscesses, borderline tumors, primary invasive stage I tumors, primary invasive stage II–IV tumors, and metastatic tumors.

Serum CA-125 assessment

Participating centers were encouraged to measure serum CA-125 levels. Some centers measured serum CA-125 levels in every patient, others did not. The decision to measure CA-125 or not was a reflection of the protocols in the different centers. It is also likely that clinicians sometimes chose not to measure serum CA-125 when they felt the ultrasound features of the mass were strongly suggestive of a benign lesion. Missing CA-125 values were accounted for using multiple imputation, see below.

Second-generation immunoradiometric assay kits for CA-125 II (27) from the following companies were used: Roche Diagnostics, Centocor, Cis-Bio, Abbott Laboratories Diagnostic Division, Bayer Diagnostics, and bioMérieux. All kits used the OC125 antibody. Serum CA-125 levels are expressed in U/mL.

Statistical analysis

Serum CA-125 levels were summarized using the median, the first and third quartile (i.e., the 25th and 75th percentile), and the range. For the whole sample and for pre- and postmenopausal patients separately, distributions of CA-125 for the 6 tumor subgroups were derived using kernel density estimation (28). Using this flexible nonparametric technique, a smooth estimate of a distribution is obtained by averaging a basic distribution, the kernel, over the data points. The smoothness of the estimated distribution is determined by the variance of the kernel. We used the "simple normal reference" method to set the kernel variance. Using the

Table 1. Serum CA-125 levels (U/mL) according to tumor pathology and menopausal status

Tumor pathology	Serum CA-125, U/mL								
	All patients ($N = 3,511$)			Premenopausal ($N = 2,134$)			Postmenopausal ($N = 1,377$)		
	n	Median (IQR)	Range	*n*	Median	Range	*n*	Median	Range
Benign	2,560	18 (11–39)	1–40,140	1,809	20	1–40,140	751	15	2–13,510
Endometrioma	713	43 (23–89)	2–3,500	683	44	4–3,500	30	33	2–844
Teratoma	402	13 (9–20)	1–288	345	14	1–288	57	11	4–30
Simple cyst/parasalpingeal cyst	281	14 (10–20)	4–512	163	15	4–512	118	12	4–220
Functional cyst	116	16 (10–25)	2–360	100	16	2–360	16	14	5–37
Hydrosalpinx + salpingitis	100	19 (12–49)	5–607	88	19	5–607	12	22	6–232
Peritoneal pseudocyst	21	16 (11–29)	8–137	18	16	8–75	3	23	8–137
Abscess	42	45 (20–123)	1–1,012	30	61	1–800	12	28	11–1,012
Fibroma	152	18 (12–41)	3–1,409	56	20	3–382	96	16	3–1,409
Serous cystadenoma	420	14 (10–21)	2–780	173	14	3–381	247	15	2–780
Mucinous cystadenoma	270	15 (10–24)	2–13,510	133	16	2–306	137	14	3–13,510
Rare benign	43	17 (11–38)	5–40,140	20	25	7–40,140	23	14	5–32,56
Malignant	951	158 (35–610)	2–38,161	325	80	4–18,055	626	222	2–38,161
Borderline, stage I	164	29 (17–76)	3–2,779	89	27	4–2,779	75	33	3–2,080
Borderline, stage II	8	165 (36–219)	15–807	3	55	24–192	5	187	15–807
Borderline, stage III–IV	14	327 (32–942)	5–12,829	11	56	5–7,863	3	1,290	499–12,829
Primary invasive, stage I	136	81 (25–289)	2–5,620	42	41	5–3,256	94	95	2–5,620
Primary invasive, stage II	47	229 (57–907)	11–6,086	13	121	18–1,686	34	328	11–6,086
Primary invasive, stage III	334	401 (160–1,183)	7–38,161	77	375	14–18,055	257	428	7–38,161
Primary invasive, stage IV	58	725 (285–2,424)	5–10,400	13	810	132–10,400	45	704	5–7,360
Rare primary invasive	70	49 (18–210)	4–1,189	37	58	6–1,189	33	36	4–814
Metastatic invasive	120	99 (25–333)	5–4,670	40	73	7–859	80	132	5–4,670

Abbreviation: IQR, interquartile range.

Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

prevalences of the 6 subgroups among pre- and postmenopausal patients, the likelihood for each tumor subgroup based on the absolute CA-125 value and menopausal status was estimated from the CA-125 distributions obtained.

Most likely, missing values for serum CA-125 occurred mainly because investigators did not consider it necessary to measure CA-125 based on the clinical picture of the patient and the ultrasound appearance of the mass. We used multiple imputation to handle the missing values in the analysis (29, 30). In multiple imputation, the missing values are estimated (i.e., imputed) multiple times to account for the uncertainty in the estimated values. Imputations were based on variables used in the present study and other variables in the data set that were related to either the level of CA-125 or to the unavailability of CA-125 (this is a binary indicator with value 1 if CA-125 is missing and 0 otherwise). Predictive mean matching regression (31) was used to generate 100 imputations of the missing values, resulting in 100 completed data sets. Each of the completed data sets was analyzed, and the results were averaged to obtain final results. The results based on multiple imputation of missing values were compared with the results based only on the patients with available CA-125 values, and the differences were described.

We used SAS version 9.2 (SAS Institute) for all analyses.

Results

Study population

The IOTA study group has collected data on 3,511 patients with at least one persistent adnexal mass: 1,066 in phase 1, 507 in phase 1b, and 1,938 in phase 2 (Supplementary Table S1). The prevalence of malignancy was 27% (25% in phase 1, 28% in phase 1b, 28% in phase 2): 35% in oncology referral centers (range over centers: 18%–57%), 11% in referral centers for ultrasonography (range over centers: 8%–17%), and 17% in public hospitals (range over centers: 8%–21%). The median age was 45 years (interquartile range: 34–58), and 39% of the patients were postmenopausal. Patients from oncology referral centers had a median age of 47 years (interquartile range: 34–59) with 43% being postmenopausal, patients from referral centers for ultrasonography had a median age of 39 years (interquartile range: 31–51) with 27% being postmenopausal, and patients from public hospitals had a median age of 44 years (interquartile range: 34-57) with 36% being postmenopausal.

Information on the serum CA-125 level was missing in 26% of the patients (in 30% in patients with a benign mass, in 9% in patients with a malignant mass), with 4 centers having missing CA-125 values in more than 30% of their cases. The rate of missing CA-125 information was similar in the 3 phases of the IOTA study (range: 22%–32%) and in the different types of centers (range: 22%–31%), with oncology referral centers having the lowest rate.

CA-125 levels according to the specific tumor pathology

Table 1 presents CA-125 levels in tumors with different pathology for all patients as well as separately for pre- and postmenopausal patients. All but 2 benign pathologies had median CA-125 levels below 20 U/mL, more specifically between 13 and 19 U/mL. Endometriomas and abscesses had median levels of 43 and 45 U/mL, respectively. Among malignant pathologies, stage I borderline tumors had the lowest CA-125 levels (median: 29 U/mL), followed by rare primary invasive tumors (median: 49 U/mL) and stage I primary invasive tumors (median: 81 U/mL). Metastatic tumors (median: 99 U/mL) had CA-125 levels similar to stage I primary invasive tumors but lower levels than higher stage primary invasive tumors (median: 229 U/mL for stage II, 401 U/mL for stage III, and 725 U/mL for stage IV) and higher stage borderline tumors (median: 165 U/mL for stage II and 327 U/mL for stage III–IV). When stratifying for menopausal status, a similar picture emerged for both pre- and postmenopausal patients. However, for benign tumors, CA-125 levels were higher in premenopausal than in postmenopausal patients, whereas for malignant tumors, CA-125 levels were higher in postmenopausal patients. As a result, the discrimination between benign and malignant tumors using serum CA-125 was superior for postmenopausal patients.

For primary invasive tumors, we computed CA-125 levels for the following histologies: serous, mucinous, endometrioid, clear cell, epithelial not otherwise specified, and nonepithelial tumors (Supplementary Table S2). Serous tumors had the highest CA-125 levels followed by epithelial not otherwise specified tumors. However, these histologic types were most often advanced stage malignancies and/or found in postmenopausal patients (Supplementary Table S2). We did not consider histology for defining the tumor subgroups to avoid small group sizes.

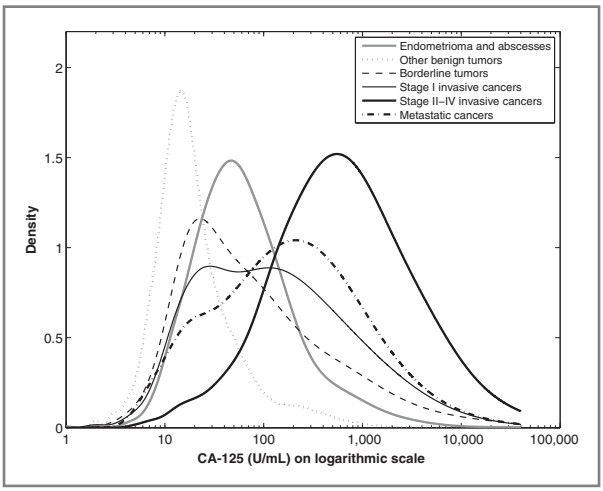

Figure 1. CA-125 distributions according to tumor subgroup for all patients.

Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

CA-125 distribution according to tumor subgroups

The estimated CA-125 distributions for endometriomas and abscesses, other benign tumors, borderline tumors, primary invasive stage I tumors (including rare primary invasive stage I tumors), primary invasive stage II–IV tumors (including rare primary invasive stage II-IV tumors), and metastatic tumors are shown in Fig. 1. The CA-125 level is shown on a logarithmic scale as it is considerably skewed. The main observation is that only 2 tumor subgroups were well separated with respect to their serum CA-125 levels: stage II–IV primary invasive tumors and benign tumors other than endometriomas or abscesses. The distributions of the remaining 4 subgroups exhibited substantial overlap with each other and also with the other 2 subgroups. This was true of both pre- and postmenopausal patients (Fig. 2).

Using the estimated distributions together with the subgroup prevalences, likelihoods for the 6 tumor subgroups were derived over the range of serum CA-125 levels. These likelihoods are visualized in Supplementary Fig. S1 for all patients and in Fig. 3 after stratification for menopausal status. For all patients, a tumor was most likely an endometrioma or an abscess if CA-125 values fell between 40 and 179 U/mL. If the CA-125 value was less than 40 U/mL, any other type of benign pathology was more probable, whereas CA-125 values above 179 U/mL made a primary invasive stage II–IV tumor the most likely diagnosis. The probability of any benign tumor exceeded that of a primary invasive stage II–IV tumor up to a CA-125 level of 236 U/mL, of any invasive tumor (primary or metastatic) up to a CA-125 level of 168 U/mL, and of any malignant tumor up to a CA-125 level of 148 U/mL.

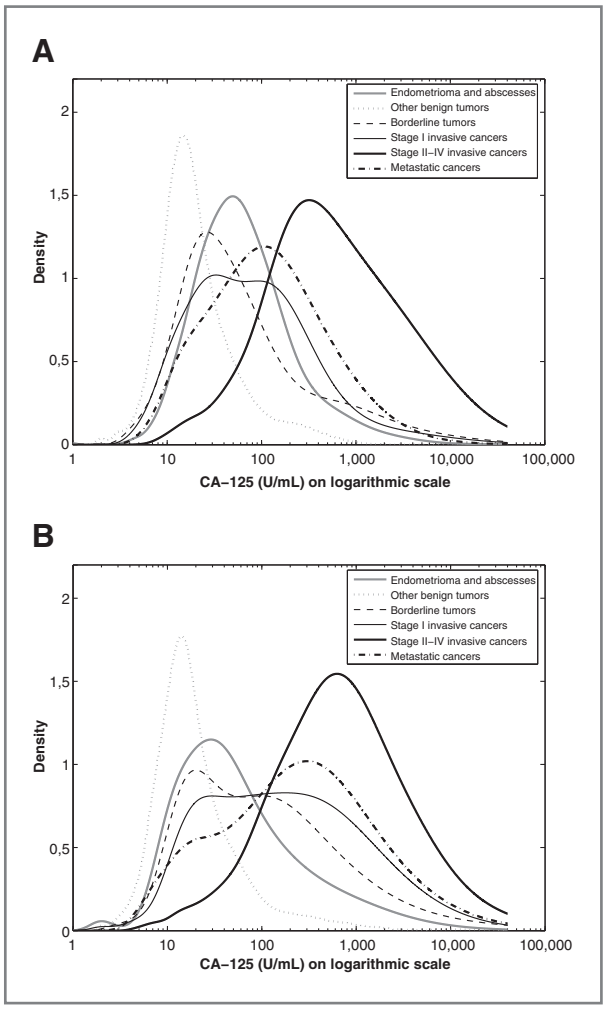

Figure 2. CA-125 distributions according to tumor subgroup for premenopausal (A) and postmenopausal (B) patients.

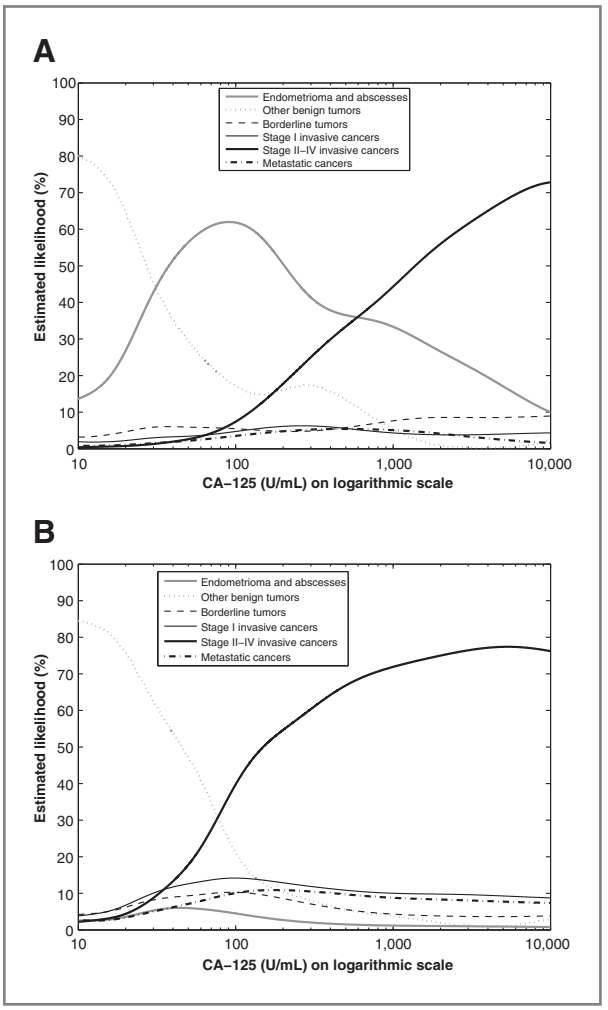

Figure 3. The likelihood of specific tumor subgroups for premenopausal (A) and postmenopausal (B) patients.

The likelihoods of the tumor subgroups according to CA-125 level were different for pre- and postmenopausal patients (Fig. 3). This is explained by differences in pathology. The prevalence of endometrioma was 32.0% in premenopausal patients versus 2.2% in postmenopausal patients, and the prevalence of invasive malignancies was 10.5% versus 39.4%. For postmenopausal

Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

Table 2. The likelihood of a specific tumor subgroup based on the CA-125 value for premenopausal patients

CA-125 value (U/mL)	Likelihood, %							
	Endometrioma or abscess	Other benign tumor	Any benign tumor	Borderline tumor	Stage I cancer	Stage II–IV cancer	Metastasis	Any malignant tumor
10	13.7	80.0	*93.7*	3.2	1.9	0.4	0.8	*6.3*
20	26.9	64.4	*91.3*	4.7	2.2	0.8	1.1	*8.7*
30	42.8	45.5	*88.3*	5.9	3.0	1.3	1.5	*11.7*
40	51.6	35.3	*86.9*	6.0	3.3	1.9	1.9	*13.1*
50	56.5	29.4	*85.9*	5.9	3.4	2.6	2.2	*14.1*
60	59.3	25.4	*84.7*	5.8	3.6	3.4	2.5	*15.3*
70	60.9	22.4	*83.3*	5.8	3.9	4.3	2.8	*16.7*
80	61.7	20.1	*81.8*	5.7	4.2	5.2	3.1	*18.2*
90	62.0	18.4	*80.4*	5.6	4.5	6.3	3.3	*19.6*
100	61.8	17.1	*78.9*	5.5	4.7	7.4	3.5	*21.1*
150	57.2	14.9	*72.1*	5.0	5.7	12.9	4.3	*27.9*
200	50.5	16.1	*66.6*	4.8	6.1	17.8	4.8	*33.4*
250	45.0	17.2	*62.2*	4.7	6.3	21.7	5.1	*37.8*
300	41.4	17.3	*58.7*	4.8	6.2	25.0	5.2	*41.3*
400	37.8	15.6	*53.4*	5.3	5.9	29.9	5.4	*46.6*
500	36.5	13.3	*49.9*	5.8	5.6	33.4	5.4	*50.1*
750	35.1	8.5	*43.6*	6.9	4.8	39.5	5.3	*56.4*
1,000	33.3	5.3	*38.7*	7.6	4.3	44.3	5.1	*61.3*
2,000	26.6	0.9	*27.5*	8.6	3.8	56.0	4.1	*72.5*
3,000	22.5	0.3	*22.8*	8.5	3.8	61.5	3.3	*77.2*
5,000	17.1	0.3	*17.4*	8.6	4.0	67.6	2.4	*82.6*
10,000	10.0	2.3	*12.3*	8.9	4.3	72.8	1.6	*87.7*

patients, the likelihood of a benign tumor exceeded that of a primary invasive stage II–IV tumor for CA-125 levels up to 82 U/mL, of any invasive tumor for CA-125 levels up to 62 U/mL, and of any malignant tumor for CA-125 levels up to 55 U/mL. For premenopausal patients, these levels were 848, 607, and 495 U/mL, respectively.

Tables 2 and 3 present reference tables with likelihood of each tumor subgroup depending on the absolute serum CA-125 level and menopausal status.

In Supplementary Table S3, results for commonly used CA-125 cutoff values to indicate malignancy are presented for all patients and for pre- and postmenopausal patients separately. The results show that the ability of CA-125 to discriminate between benign and malignant tumors was poorer in premenopausal patients. This is partly explained by the large difference in prevalence of endometriomas between pre- and postmenopausal patients.

The results based on multiple imputation of missing CA-125 values were similar to the results based on the cases with available CA-125 values. In the latter analysis, the likelihood of a malignant tumor was somewhat higher because CA-125 values were more often missing in benign tumors. The most extreme difference in percentage likelihood was 12%. The reference tables based only on the cases with available CA-125 levels are presented in Supplementary Tables S4 and S5.

Discussion

In this study, we examined the relationship between serum CA-125 levels and tumor pathology in a large series of adnexal masses. We have established reference tables to show the likelihood of a specific tumor subgroup based on the serum CA-125 value and menopausal status. This report presents graphical illustrations of the substantial overlap in CA-125 distributions between different tumor pathologies both in pre- and postmenopausal patients. CA-125 levels were higher in endometriomas and abscesses than in other benign tumors. The levels were lower in borderline tumors, stage I invasive ovarian cancers, and metastatic tumors than in stage II–IV ovarian cancers. For endometriomas and abscesses, stage I ovarian cancers, and borderline tumors, CA-125 distributions were similar. Only for higher stage ovarian cancer and benign tumors that were not endometriomas or abscesses was there a good separation of CA-125 distributions. Figs. 1 and 2 illustrate the limitations of using CA-125 as a single method for discrimination between benign and malignant tumors.

Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

Table 3. The likelihood of a specific tumor subgroup based on the CA-125 value for postmenopausal patients

CA-125 value, U/mL	Likelihood, %							
	Endometrioma or abscess	Other benign tumor	Any benign tumor	Borderline tumor	Stage I cancer	Stage II–IV cancer	Metastasis	Any malignant tumor
10	2.6	84.5	*87.1*	4.2	3.8	2.2	2.7	*12.9*
20	3.6	75.7	*79.2*	6.2	6.8	4.4	3.4	*20.8*
30	5.3	62.7	*68.0*	8.2	10.1	8.7	5.0	*32.0*
40	6.0	53.8	*59.8*	9.0	11.7	13.3	6.2	*40.2*
50	6.0	47.2	*53.1*	9.3	12.6	17.8	7.1	*46.9*
60	5.7	40.9	*46.6*	9.7	13.2	22.6	7.9	*53.4*
70	5.4	34.8	*40.3*	10.0	13.7	27.5	8.6	*59.7*
80	5.1	29.3	*34.5*	10.2	14.0	32.1	9.2	*65.5*
90	4.8	24.8	*29.6*	10.3	14.1	36.3	9.7	*70.4*
100	4.5	21.2	*25.7*	10.3	14.2	39.8	10.1	*74.3*
150	3.4	12.6	*15.9*	9.5	13.6	50.1	10.8	*84.1*
200	2.7	10.3	*13.0*	8.6	12.9	54.7	10.9	*87.0*
250	2.3	9.1	*11.4*	7.8	12.3	57.8	10.7	*88.6*
300	2.0	8.1	*10.2*	7.1	11.9	60.4	10.5	*89.8*
400	1.7	6.5	*8.2*	6.2	11.3	64.2	10.1	*91.8*
500	1.5	5.5	*7.1*	5.6	10.8	66.8	9.7	*92.9*
750	1.3	4.4	*5.7*	4.7	10.3	70.2	9.1	*94.3*
1,000	1.2	3.8	*5.0*	4.3	10.0	71.9	8.8	*95.0*
2,000	1.1	2.0	*3.1*	3.8	9.8	75.0	8.3	*96.9*
3,000	1.0	1.2	*2.2*	3.7	9.6	76.4	8.1	*97.8*
5,000	0.9	1.1	*2.0*	3.6	9.3	77.4	7.7	*98.0*
10,000	0.8	3.0	*3.8*	3.9	8.7	76.2	7.4	*96.2*

A strength of our study is that a very large number of masses from both pre- and postmenopausal patients from 21 centers in 9 countries have been collected. This means that the results should have general applicability. Moreover, our study describes the likelihood of malignancy and type of adnexal mass depending on the absolute serum CA-125 level and menopausal status.

A limitation is that information on CA-125 was missing in 26% of the masses. We have addressed this by using multiple imputation (30). An analysis including only the cases with CA-125 results available would have been based on a biased sample because in all likelihood, missing values occurred mainly because investigators did not consider serum CA-125 assessment necessary, given the clinical picture of the patient and the ultrasound appearance of the mass. This resulted in a larger proportion of missing values among patients who turned out to have a benign (30%) than a malignant tumor (9%). It is likely that the multiple imputation procedure yielded results that are closer to the truth than an analysis based on patients with available CA-125 results. Another limitation is that our study only included surgically removed adnexal masses. Therefore, our results may not be applicable to an unselected population of adnexal masses, where many masses are managed conservatively. We do believe, however, that our results are representative of adnexal masses currently considered appropriate to remove surgically.

The use of different assay kits for CA-125 assessment in different centers will have introduced some variability in the CA-125 levels (32). However, one study that compared 7 different immunoassays found that these were reliable for serum CA-125 quantification and showed highly similar diagnostic accuracy (32). Variability may also have been introduced by examining different patient populations. The use of different assay kits reflects the reality of clinical practice and is likely to lead to results that are more generally applicable and less assay dependent.

We did not consider tumor histology when defining tumor subgroups to avoid small groups. Because primary invasive malignancies of the serous type clearly had the highest CA-125 levels, the results might be different in regions where serous malignancies are less prevalent.

Our results confirm those of previous studies showing that CA-125 levels are higher in premenopausal patients than in postmenopausal patients presenting with a benign tumor but that the opposite is true of patients with a malignant tumor (9, 12, 33). This can be partly explained by differences in prevalence of endometriomas and advanced ovarian cancers. Endometriomas (with higher CA-125 values than other benign masses) are much more common in premenopausal patients, whereas advanced

Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

ovarian cancer (with higher CA-125 values than early-stage cancer) is more common in postmenopausal patients (Table 1). Even within most of the malignant pathologies listed in Table 1, the median CA-125 level was higher in postmenopausal women. This might be explained by differences in tumor spread, for example, postmenopausal patients with stage III disease may have more intra-abdominal metastases than those in premenopausal patients. Also, primary invasive cancers in postmenopausal patients were more often serous and less often mucinous or nonepithelial (see Supplementary Table S2). In healthy women, higher CA-125 levels have been reported for women who have not yet reached menopause (12). For most benign pathologies, the CA-125 levels we recorded were in agreement with this finding (Table 1). CA-125 levels may also be increased in healthy women during menstruation (12). We did not, however, record the cycle day when the blood sample for analysis of CA-125 was taken.

Simple cutoff values for CA-125 are still being used to try to discriminate between benign and malignant adnexal masses. However, there are methods that are clearly superior to CA-125 for discrimination between benign and malignant adnexal masses, for example a transvaginal ultrasound examination with subjective interpretation of ultrasound findings by an experienced ultrasound examiner (9).

To conclude, or results highlight the limitations of CA-125 to predict malignancy in ovarian masses. We have shown that CA-125 may be used in a different way. By using likelihood reference tables, we believe clinicians will be better able to interpret preoperative serum CA-125 results in patients with adnexal masses.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank the free use of a dedicated study screen developed by Astraia GmBH (Munich, Germany). The Steering Committee of the International Ovarian Tumor Analysis (IOTA) study group consists of Dirk Timmerman (University Hospitals K.U. Leuven), Lil Valentin (Skåne University Hospital), Tom Bourne (Imperial College), Antonia C. Testa (Università Cattolica di Sacre Cuore), Sabine Van Huffel (Katholieke Universiteit Leuven), and Ignace Vergote (University Hospitals K.U. Leuven).

The IOTA principal investigators are (in alphabetical order): Jean-Pierre Bernard (Maurepas, France), Artur Czekierdowski (Lublin, Poland), Elisabeth Epstein (Lund and Stockholm, Sweden), Enrico Ferrazzi (Milano, Italy), Daniela Fischerová (Prague, Czech Republic), Dorella Franchi (Milano, Italy), Robert Fruscio (Monza, Italy), Stefano Greggi (Napoli, Italy), Stefano Guerriero (Cagliari, Italy), Jingzhang (Beijing, China), Davor Jurkovic (London, UK), Fabrice Lécuru (Paris, France), Francesco PG Leone (Milano, Italy), Andrea A Lissoni (Monza, Italy), Ulrike Metzger (Paris, France), Henry Muggah (Hamilton, ON, Canada), Dario Paladini (Napoli, Italy), Alberto Rossi (Udine, Italy), Luca Savelli (Bologna, Italy), Antonia C Testa (Roma, Italy), Dirk Timmerman (Leuven, Belgium), Diego Trio (Milano, Italy), Lil Valentin (Malmö, Sweden), Caroline Van Holsbeke (Genk, Belgium), and Gerardo Zanetta (deceased; Monza, Italy).

Grant Support

This work was supported by the Research Council of the Katholieke Universiteit Leuven (B. Van Calster and S. Van Huffel): GOA Ambiorics, GOA MaNet, CoE EF/05/006 Optimization in Engineering (OPTEC), PFV/10/002 (OPTEC); Flemish Government: FWO G.0302.07 (B. Van Calster and S. Van Huffel), FWO G.0341.07 (B. Van Calster and S. Van Huffel), FWO 1.2.516.09N (B. Van Calster), IWT-TBM070706-IOTA3 (B. Van Calster, C. Van Holsbeke, S. Van Huffel, and D. Timmerman), IBBT (B. Van Calster and S. Van Huffel); Belgian Federal Science Policy Office (B. Van Calster and S. Van Huffel): IUAP P6/04 (DYSCO, "Dynamical systems, control and optimization," 2007–2011); Swedish Medical Research Council (L. Valentin): grants numbers K2001-72X 11605-06A, K2002-72X-11605-07B, K2004-73X-11605-09A and K2006-73X-11605-11-3; funds administered by Malmö University Hospital (L. Valentin); and 2 Swedish governmental grants (L. Valentin): ALF-medel and Landstingsfinansierad Regional Forskning. B. Van Calster is a postdoctoral fellow of the Research Foundation–Flanders (FWO).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 5, 2011; revised July 29, 2011; accepted September 1, 2011; published OnlineFirst September 9, 2011.

References

1. Vergote I, De Brabanter, Fyles A, Bertelsen K, Einhorn N, Sevelda P, et al. Prognostic importance of degree of differentiation and cyst rupture in stage I invasive epithelial ovarian carcinoma. Lancet 2001;357:176–82.
2. Bristow RE, Berek JS. Surgery for ovarian cancer: how to improve survival. Lancet 2006;367:1558–60.
3. Earle CC, Schrag D, Neville BA, Yabroff KR, Topor M, Fahey A, et al. Effect of surgeon specialty on process of care and outcomes for ovarian cancer patients. J Natl Cancer Inst 2006;98:172–80.
4. Yazbek J, Raju SK, Ben-Nagi J, Holland TK, Hillaby K, Jurkovic D. Effect of quality of gynaecological ultrasonography on management of patients with suspected ovarian cancer: a randomised controlled trial. Lancet Oncol 2008;9:124–31.
5. Singh AP, Senapati S, Ponnusamy MP, Jain M, Lele SM, Davis JS, et al. Clinical potential of mucins in diagnosis, prognosis, and therapy of ovarian cancer. Lancet Oncol 2008;9:1076–85.
6. Diamandis EP. Cancer biomarkers: can we turn recent failures into success? J Natl Cancer Inst 2010;102:1462–7.
7. Bast Jr RC, Klug TL, St John E, Jenison E, Niloff JM, Lazarus H, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 1983;309:883–7.
8. Jacobs I, Bast RC Jr. The CA 125 tumor-associated antigen: a review of the literature. Hum Reprod 1989;4:1–12.
9. Van Calster B, Timmerman D, Bourne T, Testa AC, Van Holsbeke C, Domali E, et al. Discrimination between benign and malignant adnexal masses by specialist ultrasound examination versus serum CA-125. J Natl Cancer Inst 2007;99:1706–14.
10. Medeiros LR, Rosa DD, da Rosa MI, Bozzetti MC. Accuracy of CA 125 in the diagnosis of ovarian tumors: a quantitative systematic review. Eur J Obstet Gynecol Reprod Biol 2009;142:99–105.
11. Cannistra SA. Cancer of the ovary. New Engl J Med 2004;351:2519–29.
12. Duffy MJ, Bonfrer JM, Kulpa J, Rustin GJS, Soletormos G, Torre GC, et al. CA125 in ovarian cancer: European Group on Tumor Markers guidelines for clinical use. Int J Gynecol Cancer 2005;15:679–91.
13. Lenhard MS, Nehring S, Nagel D, Mayr D, Kirschenhofer A, Hertlein L, et al. Predictive value of CA 125 and CA 72-4 in ovarian borderline tumors. Clin Chem Lab Med 2009;47:537–42.
14. Pauler DK, Menon U, McIntosh M, Symecko HL, Skates SJ, Jacobs IJ. Factors influencing serum CA125II levels in healthy postmenopausal women. Cancer Epidemiol Biomarkers Prev 2001;10:489–93.
15. Sevinc A, Adli M, Kalender ME, Camci C. Benign causes of increased serum CA-125 concentration. Lancet Oncol 2007;8:1054–5.

Published OnlineFirst September 9, 2011; DOI: 10.1158/1055-9965.EPI-11-0422

16. Johnson CC, Kessel B, Riley TL, Ragard LR, Williams CR, Xu JL, et al. The epidemiology of CA-125 in women without evidence of ovarian cancer in the prostate, lung, colorectal and ovarian cancer (PLCO) screening trial. Gynecol Oncol 2008;110:383–9.
17. Einhorn N, Bast RC Jr, Knapp RC, Tjernberg B, Zurawski VR Jr. Preoperative evaluation of serum CA 125 levels in patients with primary epithelial ovarian cancer. Obstet Gynecol 1986;67414–6.
18. Engelen MJ, de Bruijn HW, Hollema H, ten Hoor KA, Willemse PH, Aalders JG, et al. Serum CA 125, carcinoembryonic antigen, and CA 19-9 as tumor markers in borderline ovarian tumors. Gynecol Oncol 2000;78:16–20.
19. Timmerman D, Van Calster B, Jurkovic D, Valentin L, Testa AC, Bernard JP, et al. Inclusion of CA-125 does not improve mathematical models developed to distinguish between benign and malignant adnexal tumors. J Clin Oncol 2007;25:4194–200.
20. Valentin L, Jurkovic D, Van Calster B, Testa A, Van Holsbeke C, Bourne T, et al. Adding a single CA 125 measurement to ultrasound imaging performed by an experienced examiner does not improve preoperative discrimination between benign and malignant adnexal masses. Ultrasound Obstet Gynecol 2009;34:345–54.
21. Timmerman D, Ameye L, Fischerova D, Epstein E, Melis GB, Guerriero S, et al. Simple ultrasound rules to distinguish between benign and malignant adnexal masses before surgery: prospective validation by IOTA group. Br Med J 2010;341:c6839.
22. Timmerman D, Testa AC, Bourne T, Ferrazzi E, Ameye L, Konstantinovic ML, et al. A logistic regression model to distinguish between the benign and malignant adnexal mass before surgery: a multicenter study by the International Ovarian Tumor Analysis (IOTA) group. J Clin Oncol 2005;23:8794–801.
23. Van Calster B, Timmerman D, Lu C, Suykens JAK, Valentin L, Van Holsbeke C, et al. Preoperative diagnosis of ovarian tumors using Bayesian kernel-based methods. Ultrasound Obstet Gynecol 2007; 29:496–504.
24. VanHolsbeke C, Van Calster B, Testa AC, Domali E, Lu C, Van Huffel S, et al. Predicting malignancy of ovarian tumors: prospective evaluation of models from the IOTA study. Clin Cancer Res 2009;15: 684–91.
25. Timmerman D, Van Calster B, Testa AC, Guerriero S, Fischerova D, Lissoni AA, et al. Ovarian cancer prediction by logistic regression models: a prospective evaluation of diagnostic accuracy. Ultrasound Obstet Gynecol 2010;36:226–34.
26. Timmerman D, Valentin L, Bourne TH, Collins WP, Verrelst H, Vergote I. Terms, definitions and measurements to describe the sonographic features of adnexal tumors: a consensus opinion from the International Ovarian Tumor Analysis (IOTA) group. Ultrasound Obstet Gynecol 2000;16:500–5.
27. Kenemans P, van Kamp GJ, Oehr P, Verstraeten RA. Heterologous double-determinant immunoradiometric assay CA 125 II: reliable second-generation immunoassay for determining CA 125 in serum. Clin Chem 1993;39:2509–13.
28. Silverman BW. Density estimation for statistics and data analysis. London, UK: Chapman & Hall; 1986.
29. Little JR, Rubin D. Statistical analysis with missing data. 2nd ed. New York: Wiley; 2002.
30. Sterne JAC, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ 2009;338:b2393.
31. Schenker N, Taylor JMG. Partially parametric techniques for multiple imputation. Comput Stat Data Anal 1996;22:425–46.
32. Davelaar EM, van Kamp GJ, Verstraeten RA, Kenemans P. Comparison of seven immunoassays for the quantification of CA 125 antigen in serum. Clin Chem 1998;44:1417–22.
33. Baron AT, Boardman CH, Lafky JM, Rademaker A, Liu D, Fishman DA, et al. Soluble epidermal growth factor receptor (SEG-FR) and cancer antigen 125 (CA125) as screening and diagnostic tests for epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2005;14:306–18.

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

A Novel Approach to Predict the Likelihood of Specific Ovarian Tumor Pathology Based on Serum CA-125: A Multicenter Observational Study

Ben Van Calster, Lil Valentin, Caroline Van Holsbeke, et al.

Cancer Epidemiol Biomarkers Prev 2011;20:2420-2428. Published OnlineFirst September 9, 2011.