Evolving role of serum biomarkers in the management of ovarian cancer

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The availability of an ideal serum tumor marker would be of great clinical benefit for both the diagnosis and management of patients with epithelial ovarian cancer. Serum cancer antigen 125 assay significantly increases the diagnostic reliability of ultrasound in discriminating a malignant from a benign ovarian mass, especially in postmenopausal women, and it is the only well validated tumor marker for monitoring disease course. Several other tumor-associated antigens have been assessed, including glycoprotein antigens other than cancer antigen 125, soluble cytokeratin fragments, kallikreins, cytokines and cytokine receptors, vascular endothelial growth factor, D-dimer, and lisophosphatidic acid. This article assesses the potential diagnostic and prognostic role of these novel biomarkers, both alone and in combination with cancer antigen 125. The future for serum tumor marker research is represented by the emerging technology of proteomics, which may allow scientific advances comparable to those achieved with the introduction of monoclonal antibody technology.

Keywords: cancer antigen 125, cytokine, epithelial ovarian cancer, glycoprotein antigen, kallikrein, lisophosphatidic acid, proteomics, tumor marker, vascular endothelial growth factor

Ovarian malignancies include a heterogeneous group of tumors, represented by epithelial ovarian cancers, germ-cell tumors and sex-cord stromal tumors, with different epidemiology, histogenesis, natural history, biologic behavior and clinical course. Epithelial cancers, which account for 90% of ovarian malignancies, comprise different histologic subtypes that appear to be associated with distinct morphologic and molecular alterations [1]. High-grade serous and undifferentiated carcinomas frequently show p53 mutations and dysfunction of *BRCA1* and/or *BRCA2* genes, whereas low-grade serous carcinomas probably develop via activation of the RAS–RAF signaling pathway, secondary to either RAS or RAF mutations. Mucinous carcinomas arise via an adenoma–borderline tumor carcinoma sequence with KRAS mutations, whereas low-grade endometrioid carcinomas develop from endometriosis via mutations in the genes encoding β-catenin and phosphatase and tensin homolog (PTEN). Although the morphologic data strongly support an origin of clear-cell carcinoma from the endometriosis, there are limited data on the genetic alterations in these rare tumors.

Epithelial ovarian cancer is the leading cause of death from gynecologic cancer in Western countries [2]. The highest frequency is in the 50–59 year age group, but approximately 10–12% of cases occur in women under the age of 40 years [3]. More than two-thirds of cases are at an advanced stage at presentation, mostly due to the absence of specific symptoms and signs and the lack of reliable screening methods. The search for reliable serum tumor markers is ongoing. From a theoretical point of view, an ideal marker should have a high sensitivity and specificity in order to distinguish patients with cancer from those with benign conditions or healthy controls, and should also provide information related to tumor burden and activity. Markers such as the β-subunit of human chorionic gonadotropin (HCG) and α-fetoprotein (αFP) for nondysgerminomatous germ-cell tumors, have not yet been identified for the common epithelial ovarian cancers. However, some tumor-associated antigens, for example, cancer antigen (CA) 125, have been proposed in recent years as useful biochemical tools adjunctive to clinical, ultrasound and radiologic examinations for the diagnosis and monitoring of epithelial ovarian cancer. Hopefully, in the near future, new developments in proteomics research will provide us with more sensitive and specific markers for this malignancy (Box 1).

Cancer antigen 125

The only well-validated tumor marker for epithelial ovarian cancer is represented by CA 125, an antigenic determinant on a high-molecular-weight glycoprotein recognized by a monoclonal antibody against a human ovarian cancer cell line [4,5]. CA 125 can be also detected in normal adult fallopian tube, endometrium, endocervix and peritoneum, and *in vitro* studies have

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Box 1. Tumor markers tested in the management of epithelial ovarian cancer.

CA 125
Glycoprotein antigens other than CA 125
- CA 19–9, CA 15–3, CA 72–4
- OVX1, YLK-40
Soluble cytokeratin fragments
- CYFRA 21.1
Serine proteases/serine protease inhibitors
Human tissue kallikreins
- hK6, hK10, hK11
Serine protease inhibitors
- SLPI
Cytokines/cytokine receptors
Cytokines
- IL-6, M-CSF, IAP
Cytokine receptors
- sIL-2R, sTNF-R, s-Fas
Angiogenic factors
- VEGF, thymidine phosphorylase
Fibrin split products
- D-dimer
Lysophospholipids
- LPA
Proteomic markers

CA: Cancer antigen; CYFRA: Cytokeratin-19 fragments in serum; IAP: Immunosuppressive acidic protein; IL: Interleukin; hK: Human kallikrein; LPA: Lysophosphatidic acid; M-CFS: Macrophage-colony stimulating factor; sIL-2R: Soluble interleukin-2 receptor; SLPI: Secretory leukocyte protease inhibitor; sTNF-R: Soluble receptor for tumor necrosis factor; VEGF: Vascular endothelial growth factor; YLK: Human cartilage glycoprotein-39.

demonstrated that CA 125 secretion by human mesothelial cell monolayers can be enhanced by the inflammatory cytokines interleukin (IL)-1, tumor necrosis factor (TNF)-α or lipopolysaccharide from *Escherichia coli* [6]. Nonmucinous epithelial ovarian cancers express this antigen more frequently than mucinous ones [7]. High serum CA 125 levels can be measured in approximately half of patients with early stage epithelial ovarian cancer and in more than 90% of those with advanced disease [4,5,7]. However, the specificity of the antigen is not optimal, since high CA 125 levels can sometimes be detected in patients with benign gynecologic conditions, such as endometriosis and pelvic inflammatory disease [4,7]; benign nongynecologic conditions, such as hepatitis, pancreatitis and renal failure [4,5]; nonepithelial ovarian cancers [8]; extra-ovarian gynecologic malignancies, such as endometrial cancer [4,9] and cervical adenocarcinoma [10]; and nongynecologic malignancies, including lung cancer [11] and non-Hodgkin's lymphoma [12]. For instance, an association between serosal involvement and elevated serum CA 125 has been documented in patients with lymphoma, thus leading to the speculation that elevated CA 125 levels may be associated with bulky disease. Lymphoma cells themselves do not express CA 125, but release cytokines that can in turn stimulate mesothelial cells to produce and secrete this antigen [7,12].

Screening & diagnosis of epithelial ovarian cancer

A woman with a suspicious ovarian mass will undergo a laparoscopic or laparotomic assessment. For a screening test, a positive predictive value (PPV) of 10% is considered to be acceptable, corresponding to one cancer for every ten surgical procedures [13]. Since ovarian cancer has a prevalence of one case/2500 women older than 50 years, a screening test with a specificity greater than 99.7% will be needed to achieve a PPV of 10%, with a sensitivity of 67% [5]. Bast and colleagues reported that serum CA 125 elevations may precede advanced ovarian cancer detection by 10–12 months and, therefore, monitoring CA 125 could allow the identification the tumor long before it is clinically apparent [14]. Several factors can influence serum CA 125 levels in healthy postmenopausal women, as emerged in an analysis of 18,748 subjects who participated in a British ovarian cancer screening trial and who were not found to develop an ovarian cancer during the 12-year follow-up period [15]. Woman's age, age at menarche, age at menopause, race, smoking habits, caffeine consumption, history of a previous ovarian cyst, prior hysterectomy or prior cancer diagnosis other than ovarian cancer were significant predictors of baseline CA 125 levels, whereas parity, use of hormone replacement therapy, previous unilateral oophorectomy and previous oral contraceptive use were not. In any case, even in postmenopausal women, the specificity of serum CA 125 is much lower than 99.7%, and therefore CA 125 assay alone is not an adequate screening test for ovarian cancer, and a pelvic and/or transvaginal ultrasound has often been performed as a second-line test [5,16]. However, a systematic review of 17 prospective cohort studies and three pilot, randomized, controlled trials demonstrated that screening tests with CA 125 and ultrasound still obtain unsatisfactory PPVs, resulting in healthy women being recalled and a false-positive rate of 0.01–5.8% [17]. Of every 10,000 women participating in an annual screening program with CA 125 for 3 years, 800 will have an ultrasound

scan due to an elevated CA 125, 30 will undergo a surgical procedure due to an abnormal ultrasound and six will have ovarian cancer detected at surgery. Therefore, there is insufficient evidence to support the introduction of such a screening program in the asymptomatic general-risk postmenopausal population, since screening is associated with increased rates of surgery and patient anxiety and does not appear to reduce ovarian cancer morbidity or mortality. Better methods to detect and screen for this malignancy in all women, but especially in high-risk *BRCA1/2* mutation carriers, are urgently needed [18].

The treatment of an ovarian cyst depends on its nature, and an accurate preoperative discrimination of malignant versus benign mass is therefore a challenge of crucial importance [19]. As far as this differential diagnosis is concerned, serum CA 125 assay has a sensitivity for epithelial ovarian cancer ranging from 56–100%, with a specificity ranging from 60–92%, according to the selected cut-offs [4,7,20], and significantly increases the diagnostic reliability of ultrasound, especially in postmenopausal women [21–25]. For instance, in a study by Schutter and colleagues, the accuracy in discriminating a malignant from a benign pelvic mass was 76% for pelvic examination, 74% for transvaginal ultrasound and 77% for CA 125 assay taking 35 U/ml as cut-off, and no cancer was found in women in whom all three examinations were negative [22]. Logistic regression models based on different biochemical and ultrasonographic parameters may aid in the differential diagnosis of ovarian masses [26,27].

The American College of Obstetricians and Gynecologists (ACOG) and the Society of Gynecologic Oncologists (SGO) jointly published guidelines for referring women with pelvic masses suspicious for ovarian cancers to gynecologic oncologists, which are based on patient age, CA 125 level, physical findings, imaging study results and a family history of breast and ovarian cancer in a first-degree relative (Table 1) [28]. Only one criterion from the respective pre- or postmenopausal list is needed to recommend referral to a gynecologic oncologist. Im and colleagues performed a chart review of factors included in the guidelines of 1035 surgically evaluated women with pelvic masses at different tertiary care centers during a 12-month interval [29]. The referral guidelines correctly identified 70% of the ovarian cancer patients in the premenopausal group and 94% of those in the postmenopausal group. However, the PPV was only 33.8% for premenopausal women and 59.5% for postmenopausal women; therefore, these guidelines tended to over-refer women with benign masses, probably due to the large number of cases with a positive family history of breast and ovarian cancer in the study population. Aiming to explore models that might increase the PPV of the referral criteria, Im and colleagues reassessed their data, excluding the criterion of family history of breast or ovarian cancer in all women and the criterion of nodular or fixed pelvic mass on gynecologic examination in postmenopausal women, and taking the threshold value as 50 U/ml for CA 125 assay in premenopausal women [29]. Using these modified criteria, 85% of the premenopausal and 90% of the postmenopausal women with ovarian cancer would have been correctly detected for referral to a gynecologic oncology center.

Preoperative serum CA 125 level may be predictive of cytoreducibility in patients with epithelial ovarian cancer. Eltabbakh and colleagues assessed 72 patients with advanced ovarian or primary peritoneal carcinoma who underwent debulking surgery, and found that women with preoperative serum CA 125 levels of 500 U/ml or less were 7.9-times more likely to achieve complete cytoreduction than women with preoperative antigen values above 500 U/ml ($p < 0.001$) [30]. Conversely, there was no clear relationship between early postoperative drop in CA 125 levels and the size of residual disease [31–33]. In the authors' experience, the postoperative decline of serum CA 125 was significantly higher in women who were optimally cytoreduced compared with those who were not [33], but this parameter did not allow for discrimination between these two groups of women with 100% accuracy.

Monitoring of response to chemotherapy & follow-up of epithelial ovarian cancer patients

Serum CA 125 kinetics during early chemotherapy correlate with response to treatment and survival of patients with ovarian cancer [34–37]. In the authors' experience with 225 patients with advanced disease who received postoperative platinum-based chemotherapy, serum CA 125 before the third cycle of chemotherapy was an independent prognostic variable for the chance of achieving a pathologic complete response (CR) at second-look surgery, whereas serum CA 125 half-life during early chemotherapy was

Table 1. Guidelines for referring a pelvic mass suspicious for ovarian cancer to a gynecologic oncologist.

Criteria	Premenopausal (<50 years)	Postmenopausal (≥50 years)
SGO/ACOG criteria	CA 125 > 200 U/ml	CA 125 > 35 U/ml
	Ascites	Ascites
	Abdominal/distant metastases	Abdominal/distant metastases
		Nodular/fixed pelvic mass
	Family history of breast/ovarian cancer in a first-degree relative	Family history of breast/ovarian cancer in a first-degree relative
Im and colleagues' criteria	CA 125 > 50 U/ml	CA 125 > 35 U/ml
	Ascites	Ascites
	Abdominal/distant metastases	Abdominal/distant metastases

ACOG: American College of Obstetricians and Gynecologists; CA: Cancer antigen; SGO: Society of Gynecologic Oncologists.
Modified from [28] and [29].

an independent prognostic variable for both the chance of achieving a pathologic CR and survival (Table 2) [34]. In a series of 50 patients with advanced serous epithelial ovarian cancer treated with neoadjuvant platinum-based combination chemotherapy, Tate and colleagues calculated the CA 125 regression coefficient using all antigen levels measured from the first day of chemotherapy until the day of CA 125 normalization or the day of surgery, and found that this parameter significantly correlated with overall survival (OS) at univariate analysis [37]. In fact, the 3-year OS was 70.5% for patients with a regression coefficient of -0.039 or greater and 43.3% for those with a lower regression coefficient ($p = 0.012$).

In vitro and *in vivo* effects of taxanes on CA 125 expression and release by ovarian cancer cells have been long debated. Marth and colleagues found that paclitaxel and docetaxel increased CA 125 expression in three ovarian cancer cell lines constitutively expressing this tumor marker, and that the taxane-mediated induction of antigen was dependent on intact protein and RNA biosynthesis [38]. Conversely, an *in vitro* study by Bonfrer and colleagues, on two different ovarian cancer cell lines exposed to paclitaxel, detected that CA 125 levels in the culture medium were significantly related to cell numbers and, consequently, to the response of the cell line to the drug [39]. As for clinical settings, some authors reported that CA 125 regression with paclitaxel-containing regimens was slower than that with nonpaclitaxel-based regimens [40], whereas others found that changes in serum CA 125 were a very good predictor of response to paclitaxel-based chemotherapy [41,42]. For instance, in a recent retrospective investigation of 71 advanced epithelial ovarian cancer patients who received postoperative paclitaxel/platinum-based chemotherapy, serum CA 125 half-life during early chemotherapy was an independent prognostic variable for the chance of achieving a CR to treatment, as well as for progression-free survival (PFS) and OS (Table 2) [42].

Table 2. Serum CA 125 half-life during early chemotherapy in advanced epithelial ovarian cancer patients with elevated CA 125 levels before starting chemotherapy.

Chemotherapy regimen	N	Median CA 125 $T_{½}$ (days)	Correlation to CR multivariate analysis p-value	Correlation to OS multivariate analysis p-value
Platinum-based chemotherapy†	225	25	0.004	0.007
Paclitaxel/platinum-based chemotherapy‡	71	14	0.023	0.0181

CA: Cancer antigen; CR: Complete response; OS: Overall survival; $T_{½}$: Half-life.
Modified from †[34] and ‡[42].

Elevated CA 125 values at the time of second-look surgery are strong predictors of persistent disease, whereas normal antigen levels can be associated with both negative and positive second-look findings [4,20,43].

Changes in CA 125 levels correlate with regression, stability and progression of disease in 87–94% of instances and, moreover, rising antigen levels may precede the clinical detection of recurrence in 56–94% of cases, with a median lead time of 3–5 months [4,20,43–46]. Rustin and colleagues found that a doubling of serum CA 125 from the upper limit of normal (>30 U/ml) correctly predicted disease progression in patients on follow-up, and that when the doubling was confirmed by a second sample there was only one (1.2%) false-positive prediction of progression among 86 patients [46]. The same authors observed that definitions based on a 50 or 75% decrease in CA 125 levels accurately predicted which drugs were active in Phase II trials for recurrent disease and worthy of further studies [47]. The Gynecologic Cancer Intergroup (GCIG) recently proposed a simpler response definition based on just a 50% decrease in serum CA 125 [48], that was found to be a better prognostic tool than the classical Response Evaluation Criteria In Solid Tumor (RECIST) [49] in a retrospective study on patients who received second-line chemotherapy for recurrent ovarian cancer [50]. In detail, this study examined 68 patients with disease measurable by RECIST; those with solid tumors assessed by computed tomography (CT) scan (>10 mm) or by ultrasound (>20 mm), and assessable by the GCIG CA 125 response criteria, for example, with two pretreatment samples at least twice the upper limit of normal (35 U/ml) and at least two additional samples after the start of salvage treatment with topotecan or paclitaxel plus carboplatin. CA 125 response criteria were two- to three-times better than RECIST at predicting survival and, moreover, the former, but not the latter, were independent prognostic variables for survival.

Other tumor-associated antigens

From a theoretical point of view, the concomitant assay of tumor markers other than CA 125 could play a role if they are able to detect some carcinomas missed by CA 125 (i.e., they improve sensitivity), rule out false positives (i.e., they improve sensitivity), or detect the same cancers earlier. Different tumor-associated antigens have been assessed in patients with ovarian cancer in recent years.

Glycoprotein antigens other than CA 125

Tumor-associated glycoprotein antigens other than CA 125, such as CA 19.9, CA 15.3 and CA 72.4, first identified in gastrointestinal or breast cancers, have also been detected in the sera of patients with epithelial ovarian cancer [7,20,43,44]. In the authors' study, elevated CA 19.9 levels (>40 U/ml) were measured in 35.6% of 90 patients with this malignancy: 83.3% of patients with mucinous and 28.2% of those with nonmucinous tumors [7]. Although the overall sensitivity of CA 19.9 assay is unsatisfactory, the antigen is very sensitive for mucinous tumors, which often fail to express CA 125. Serum CA 15.3 was greater than 32 U/ml in 57.1% of ovarian cancer patients: 63.9% of patients with nonmucinous and 16.7% of those with mucinous tumors; and serum CA 72.4 was greater than 3.8 U/ml in 70.7% of ovarian cancer patients, with no difference according to the histologic types in terms of sensitivity.

As for the monitoring of patients with histologically proven epithelial ovarian cancer, changes in serum levels of CA 19.9, CA 15.3 and CA 72.4 correlated with disease course in more than 70% of cases [43]. Taking into consideration patients with elevated preoperative levels of both CA 125 and one or more of the other antigens, the authors observed that serum CA 125 correlated with disease course better than the other markers and, moreover, serum CA 125 usually increased earlier or in a higher percentage of cases compared with the other markers before the clinical detection of progressive disease. Therefore, in patients with positive CA 125 assay at diagnosis, the concomitant determination of CA 19.9, CA 15.3 or CA 72.4 did not offer any additional benefit, whereas the serial measurements of these other antigens may represent an interesting biochemical tool for monitoring patients with a negative CA 125 assay.

Elevated levels of OVX1, a modified Lewis determinant on a high-molecular-weight mucin, have been found in patients with early stage ovarian cancer, and a concomitant OVX1 assay appeared to increase the sensitivity of the CA 125 assay for this malignancy [51,52]. Moreover, raised OVX1 levels have been measured in a fraction of patients with normal CA 125 levels and persistent disease after first-line chemotherapy [53].

Human cartilage glycoprotein-39 (YKL-40), a glycoprotein belonging to the chitinase protein family, has been detected in sera from ovarian cancer patients [54–56]. In a study by DuPont and

colleagues, elevated preoperative levels of YKL-40 (≥62 ng/ml) and CA 125 (>35 U/ml) were measured in 20 (65%) and 11 (35%), respectively, of 31 stage I–II ovarian cancer patients and, moreover, early stage patients with YKL-40 values of more than 80 ng/ml had a worse clinical outcome compared with those with lower antigen levels [56]. In fact, among the 29 patients with a long-term follow-up, recurrent disease developed in 71% of the 14 patients with preoperative YKL-40 levels greater than 80 ng/ml, compared with none of the 15 patients with YKL-40 levels lower than 80 ng/ml. Similarly, in a series including 47 patients with stage III epithelial ovarian cancer, patients with high YKL-40 levels (>130 μg/l) had significantly shorter survival ($p = 0.0003$) than those with normal YKL-40 levels, and at Cox analysis, YKL-40 assay was found to be an independent prognostic factor for survival [55]. Therefore, this glycoprotein appears to be very promising for both diagnostic and prognostic purposes.

Soluble cytokeratin fragments

Soluble forms of fragments of cytokeratins, which represent important structural elements of the cytoskeleton, have been identified in sera from patients with several malignancies, including epithelial ovarian cancer [57–59]. The serum cytokeratin-19 fragments in serum (CYFRA) 21–1 assay, which detects serum fragments of cytokeratin 19, can discriminate malignant from benign ovarian masses but does not offer additional information to CA 125 assay alone. In the authors' experience, preoperative serum CYFRA 21–1 assay was predictive of response to chemotherapy but not prognostic of survival [59]. Conversely, in the series by Templer and colleagues, elevated pretreatment CYFRA 21–1 levels were significantly related to poor disease-free survival and OS [58].

Serine proteases & serine protease inhibitors

Human tissue kallikreins

The human tissue kallikreins (KLKs), low-molecular-weight serine proteases involved in tumor invasion and metastasis, are encoded by a gene family consisting of 15 genes mapped at the same chromosomal locus and sharing significant homology at both the nucleotide and protein level, and genomic organization [60,61]. All KLKs are initially synthesized as preproenzymes, and are then proteolytically processed to yield proenzymes and ultimately active enzymes. The best known KLK is human KLK (hK) 3, which represents the best biomarker for prostate cancer (prostate-specific antigen [PSA]). Many members of the human-tissue KLK family are overexpressed in ovarian cancer and have a potential role as diagnostic and/or prognostic markers for this malignancy [62–70]. For instance, 84.8% of 66 ovarian and peritoneal carcinoma tissue samples showed elevated expression of KLK 10 mRNA [63]. Similarly, increased expression of *KLK6* has been reported in 83.7% of 80 ovarian tumor samples and, more interestingly, in several early stage and low-grade tumors [70]. Moreover, elevated levels of hK6 protein were found in benign epithelia coexisting with borderline and invasive tumors, thus suggesting that *KLK6* overexpression is an early phenomenon in ovarian carcinogenesis. Other studies on ovarian cancer have demonstrated that the expression of *KLK4* [65,66], *KLK5* [67] and *KLK15* [68] were associated with a more aggressive course, whereas the expression of *KLK9* [64], *KLK11* [69] and *KLK14* [62] correlated with longer PFS and OS.

The presence of more than one mRNA form for the same gene is common among KLKs, and many of the KLK splice variants may hold clinical relevance. In fact, Youssef and colleagues recently identified a new alternatively spliced variant of *KLK5*, termed KLK5-splice variant 2, which appears to be a potential biomarker for breast and ovarian cancers [71].

High serum levels of hK6 [72,73], hK10 [74] and hK11 [75] have been detected in more than 50% of patients with epithelial ovarian cancer, and these KLKs are currently under evaluation as useful serum markers for this malignancy.

Diamandis and colleagues reported that serum hK6 was slightly less sensitive but more specific than CA 125 for ovarian cancer, and that hK6 assay increased the sensitivity of CA 125 by 12 or 13% at 90 or 95% specificity cut-offs, respectively, for both markers (Table 3) [73]. The same authors found that patients with high preoperative hK6 levels (>4.4 μg/l) had a shorter PFS (relative risk [RR]: 4.86; 95% confidence interval [CI]: 1.10–21.4; $p = 0.036$) and a shorter OS (RR: 5.08; 95% CI: 1.07–23.69; $p = 0.038$). KLK6 is also highly expressed in uterine papillary serous carcinoma, and hK6 protein is found in the serum of patients with this malignancy [76].

Elevated hK10 concentrations have been found in blood samples collected preoperatively from patients with ovarian cancer, but not from those with benign gynecologic disease [74]. With a cut-off point of 700 ng/l (corresponding to a

Table 3. Sensitivity of serum hK6 for ovarian cancer.

Marker	Stage	N	SE at 90% SP	SE at 95% SP
hK6	I–IV	124	58%	53%
CA 125	I–IV	124	60%	56%
hK6 or CA 125	I–IV	124	72%	69%
hK6	I–II	43	26%	21%
CA 125	I–II	43	30%	26%
hK6 or CA 125	I–II	43	42%	37%

CA: Cancer antigen; hK: Human kallikrein; SE: Sensitivity; SP: Specificity.
Modified from [73].

specificity of 90%), the sensitivity for ovarian cancer was 54% and, interestingly, approximately 35% of CA 125-negative ovarian cancer patients were hK10-positive. High serum hK10 was significantly related to advanced stage, serous histotype, high histologic grade, large residual disease, lack of response to chemotherapy and poor survival.

Raised serum hK11 has been detected in 70% of women with ovarian cancer, and the serum assay of this biomarker might aid in the diagnosis and monitoring of this malignancy [75].

Serine protease inhibitors

The expression of serine protease inhibitors in tumor samples is often associated with a poor prognosis, and there is a growing body of evidence that these substances promote the tumorigenic metastatic potential of cancer cells [77]. Secretory leukocyte protease inhibitor (SLPI) is a serine protease inhibitor expressed in several human carcinomas, including epithelial ovarian cancer [78]. Serum SLPI was found to be significantly elevated in patients with ovarian cancer compared with those with benign ovarian cysts or healthy women, and in a preliminary study, the combination of SLPI (cut-off: 50 ng/ml) and CA 125 (cut-off: 30 U/ml) had a sensitivity of 95%, a specificity of 100%, a PPV of 100% and a negative predicitive value (NPV) of 89% in differentiating malignant from benign ovarian cyst [79].

Cytokines

Cytokines are mainly produced by monocyte–macrophages and lymphocytes, but a constitutive production of cytokines and cytokine receptors by human ovarian cancer cell lines and fresh tumor biopsy material has also been demonstrated.

Elevated serum levels of cytokines, such as IL-6 [80–83], macrophage colony-stimulating factor (M-CSF) [51,52,83–85], immunosuppressive acidic protein (IAP) [83,86] and cytokine receptors, such as soluble IL-2 receptor (sIL-2R) [82,87–90] and soluble TNF receptors (sTNF-R) [91–94], have often been detected in patients with epithelial ovarian cancer.

The addition of the serum IL-6 assay did not improve the sensitivity of CA 125 assay, but an elevated preoperative IL-6 level was an independent poor prognostic factor for OS in a series of 114 patients with epithelial ovarian cancer [80]. Conversely, serum M-CSF could improve the diagnostic reliability of serum CA 125 for this malignancy [51,52,85]. When combining serum preoperative CA 125, CA 72–4, CA 15.3, and M-CSF, using mixtures of multivariate normal distributions, the sensitivity for early stage ovarian cancer was found to be 45% for CA 125; 67% for CA 125 and CA 72–4; 70% for CA 125, CA 72–4 and M-CSF; and 68% for all four markers [85]. Serial M-CSF levels correlated significantly with the disease course, but the concomitant determination of serum M-CSF appeared to add little to CA 125 assay alone in the monitoring of patients [84]. Pretreatment serum IAP greater than 1100 µg/ml was an independent poor prognostic variable for OS in a series of 80 ovarian cancer patients, with a RR of death of 2.99 (95% CI: 1.43–6.07; $p < 0.05$) and, therefore, IAP assay could represent a useful biochemical tool for the prognostic characterization of these patients [86].

The clinical relevance of serum sIL-2R assay for the early detection and prognostic evaluation of epithelial ovarian cancer is still controversial [87,89,90], but serial sIL-2R measurements seem to be of limited value for the management of this malignancy [87].

The serum assay of sTNF-R gives no additional information over CA 125 for discriminating malignant from benign ovarian masses [93], whereas it could have clinical relevance for prognostic purpose [91,92,94]. In the authors' experience, preoperative sTNF-R levels were significantly higher in ovarian cancer

patients who died of the disease or were alive with clinical evidence of disease compared with those without clinical evidence of disease 2 years after initial surgery [92]. In a recent Gynecologic Oncology Group (GOG) study, serum pretreatment CA 125 and sTNF-R levels were predictive of PFS, but not of OS, after adjusting for patient age, histologic subtype and extent of disease [94].

Fas, a member of the TNF-R superfamily, and its specific ligand (FasL) exist in membrane-bound and soluble forms [95,96]. The soluble forms have been observed in different tumors, but their clinical significance has not yet been clarified. Whereas the interaction between FasL and membrane-bound Fas induces apoptosis in sensitive cells, the binding of soluble Fas (sFas) to FasL prevents FasL-membrane-bound Fas linkage and blocks Fas-mediated apoptosis [95,97]. The suppression of apoptosis contributes to carcinogenesis, as well as to resistance to chemotherapy [98]

Elevated serum sFas levels have been detected in ovarian cancer patients, and serum sFas assay appeared to be able to discriminate malignant from benign ovarian masses [98,99]. In an international case–control study including 138 women with epithelial ovarian cancer diagnosed between 2 months and 13.2 years after the initial blood collection, and 263 control women, serum sFas levels were similar in women subsequently diagnosed with ovarian cancer (median: 6.5 ng/ml; range: 4.4–10.2) and in controls (median: 6.8 ng/ml; range: 4.5–10.1), and therefore serum sFas did not appear to be a suitable marker for the identification of women at an increased risk of this malignancy [100]. On the other hand, serum sFas assay seems to have a prognostic relevance for patients with ovarian cancer, since increased pretreatment sFas levels are significantly related to poor survival [98,99].

Inhibin is a member of the transforming growth factor (TGF)-β family of cytokines, consisting of two heterologous subunits (forming disulfide-linked dimers, inhibin A and B), that is released primarily by the ovaries and plays an essential role in regulating pituitary follicle-stimulating hormone (FSH) secretion by a negative feedback mechanism [101]. Serum inhibin assay could be especially useful after the menopause, when ovarian production of inhibin decreases to negligible levels. In an early paper by Healy and colleagues, elevated serum inhibin was detected in 82% of the 22 patients with mucinous ovarian carcinomas, 16.4% of the 91 patients with non-mucinous ovarian carcinomas, 100% of the three patients with granulosa cell tumors and 19% of the 27 patients with other ovarian tumors [102]. Some authors reported that the concomitant determination of serum inhibin and CA 125 had a greater diagnostic reliability than serum CA 125 alone for ovarian cancer [101,103–106]. High preoperative serum inhibin A level appeared to be an independent poor prognostic variable for disease-free survival and OS in 44 postmenopausal women with this malignancy [107].

Angiogenic factors

Vascular endothelial growth factor (VEGF) has been detected in surgical samples from primary and metastatic epithelial ovarian cancer [108,109], as well as in sera from patients with this malignancy [110–116]. VEGF levels are markedly elevated in patients with advanced-stage or poorly differentiated tumors or with large-volume ascites (>500 ml), compared with those with early stage and well-differentiated tumors or with small-volume ascites [115]. The reanalyses of pooled data of 314 ovarian cancer patients included in four studies [111–114] demonstrated that high pretreatment serum VEGF was associated with a shortened OS at both univariate and multivariate analysis [Hefler *et al.*, unpublished data]. In the subset of patients with stage I disease, higher tumor grade and serum VEGF were the only independent prognostic variables for OS, and patients with serum VEGF levels of 389 pg/ml or greater had a ninefold increased risk of cancer-related death. VEGF assay may be useful for the biochemical surveillance of ovarian cancer patients, since VEGF levels decrease after cytoreductive surgery and increase when the tumor relapses [110,115]. In a recent study by Alvarez Secord and colleagues, including 62 patients who completed first-line chemotherapy, high serum VEGF before second-look surgery was predictive of persistent disease and prognostic of poor survival [116].

As for other angiogenic factors, platelet-derived endothelial-cell growth factor (PD-ECGF) has been found to be expressed more in malignant than in benign ovarian tumors [117]. PD-ECGF is homologous to thymidine phosphorylase [118], and this enzyme activity appears to be essential for the stimulatory effect of PD-ECGF on angiogenesis [119]. In a recent investigation, a significantly high thymidine phosphorylase activity was detected in both tumor tissues and sera from ovarian cancer patients [120]. A positive correlation between tumor tissue and serum

Table 4. Lysophosphatidic acid in epithelial ovarian cancer.

Study	Patients with epithelial ovarian cancer				Controls (n)	p-value	Ref.
	Stage I (n)	*Stage II–IV (n)*	*Recurrent disease (n)*				
Xu *et al.*	10	24	14		48		[132]
Total LPA μmol/l (median, range)	2.4 (1.0–32.3)	5.2 (1.8–43.1)	4.1 (1.4–33.8)		0.1 (<0.1–6.3)	<0.001	
	Patients with epithelial ovarian cancer				Controls (n)	p-value	
	Stage I (n)	*Stage II (n)*	*Stage III (n)*	*Stage IV (n)*			
Suthpen *et al.*	7	3	31	4	27		[136]
Total LPA μmol/l (mean, standard deviation)	2.57 (0.94)	2.15 (0.71)	2.93 (1.77)	1.97 (0.27)	0.90 (0.43)	<0.0001	

Modified from [132] and [136].

thymidine phosphorylase activity was observed, and serum enzyme activity was higher in advanced- than in early stage ovarian cancer patients, but the difference did not reach statistical significance. Although thymidine phosphorylase is not a secretory enzyme, it can nevertheless pass from disintegrating tumor cells into the circulation. The role of serum thymidine phosphorylase as a biomarker of ovarian cancer should be further investigated.

Fibrin split products

Malignant cells secrete procoagulant and fibrinolytic factors that ultimately result in the formation of fibrin split products known as D-dimer (DD) fragments, which have been found to be markedly elevated in the sera of patients with different tumors, including epithelial ovarian cancer [116,121–126]. In the authors' study of 121 patients with surgically assessed ovarian masses, the logistic regression showed that both DD assay (cut-off: 416 ng/ml) and CA 125 assay (cut-off: 65 U/ml) were significant predictive variables for malignancy ($p = 0.0001$), and the sensitivity, specificity, PPV and NPV of the tests in differentiating malignant from benign ovarian disease were as follows: 76.8, 93.8, 91.5 and 82.4%, respectively, for CA 125; 94.6, 76.9, 77.9 and 94.3%, respectively, for the combination CA 125 or DD; and 73.2, 100, 100 and 81.3%, respectively, for the combination of CA 125 and DD [121]. Therefore, the combined determination of the two antigens appeared to be a useful diagnostic tool. It is worth noting that DD and CA 125 were above the respective cut-offs in 73.3 and 33.3%, respectively, of patients with stage I ovarian cancer.

The prognostic relevance of DD assay is still uncertain: elevated pretreatment DD levels were associated with a poor OS in some series [122,125] but not in others [123]. Moreover, DD levels before second-look surgery were neither predictive of response to chemotherapy nor prognostic of survival [116]. The concomitant determination of DD and CA 125 did not improve the reliability of CA 125 alone in the monitoring and follow-up of patients with epithelial ovarian cancer [126].

Lysophosphatidic acid

A novel member of the TNF cytokine family, termed TNF-related apoptosis-inducing ligand (TRAIL), enhances apoptosis in a wider range of cancer cells than FasL [127], and a high expression of the *TRAIL* gene measured with quantitative real-time polymerase chain reaction (PCR) has recently been associated with prolonged survival in advanced ovarian cancer patients [128]. Lysophosphatidic acid (LPA), a glycerophospholipid released by activated platelets during coagulation [129], has recently been found to be produced by ovarian cancer cells [130–132]. This lipid prevents TRAIL-induced apoptosis mainly via activation of the phosphatidylinositol 3-kinase (PI3K)–Akt signaling pathway and Bad phosphorylation, thus suppressing caspase activation [133]. Moreover, LPA induces translocation of Fas from the cell membrane to the cytosol, thus preventing ovarian cancer cell destruction by FasL-bearing immune cells [134], and enhances ovarian cancer cell invasiveness via increased expression of IL-8 and matrix-metalloproteinase (MMP)-7, as shown by *in vitro* studies determining the ability of cells to invade a synthetic basement membrane [135].

Elevated LPA levels have been measured with gas chromatography [132] and electrospray ionization mass spectrometry [136] in blood samples from ovarian cancer patients, even from those

Table 5. Tumor markers in epithelial ovarian cancer: clinical use.

Biomarker	Diagnosis	Prognosis	Follow-up
CA 125	++	+++	+++
CA 19–9*	+	+	++
OVX 1	+	?	+
YLK-40	++	++	?
CYFRA 21.1	+	±	+
hK6	++	++	?
hK10	++	++	?
IL-6	+	+++	?
M-CFS	++	±	+
VEGF	+	+++	+
D-dimer	++	±	+
LPA	++	?	?
Proteomic markers	+++	?	?

**For mucinous tumors.*

CA: Cancer antigen; CYFRA: Cytokeratin-19 fragments in serum; hK: Human kallikrein; IL: Interleukin; LPA: Lysophosphatidic acid; M-CFS: Macrophage-colony stimulating factor; VEGF: Vascular endothelial growth factor; YLK: human cartilage glycoprotein-39.

with early disease, which supports the utility of LPA as biomarker of this malignancy (Table 4). For instance, with a cut-off of 1.3 μmol/l, Xu and colleagues measured raised total LPA levels in 98% of 48 patients with ovarian cancer: 90% of the 10 patients with stage I disease, 100% of the 24 patients with stage II–IV disease and 100% of the 14 patients with recurrent disease. In contrast, 23.5% of the 17 patients with benign gynecologic disease and 10.4% of 48 healthy controls had raised LPA levels [132]. Using a receiver-operating characteristics-derived cut-off value of 1.5 μmol/l, Sutphen and colleagues found that LPA assay had a sensitivity of 91.1% and a specificity of 92.6% for ovarian cancer [136]. On the other hand, another study performed with a liquid chromatography/mass spectroscopy assay failed to detect significant differences in serum LPA levels between ovarian cancer patients and controls [137]. The conflicting results reported in the literature may partially be due to methodologic differences in sample collection, processing and lipid analyses. Large studies with well-defined laboratory methods are warranted to assess the clinical relevance of LPA assay, both alone and in combination with other markers, including proteomic markers and algorithms of changes in CA 125 levels over time, for screening, diagnosis and monitoring of epithelial ovarian cancer [136].

Proteomic markers

Low-molecular-weight serum-protein profiling might reflect the underlying pathologic state of an organ such as the ovary [138]. Matrix-assisted and surface-enhanced laser desorption and ionization time-of-flight mass spectroscopy can simultaneously analyze the expression levels of many proteins in clinical samples [138–147]. Highly sophisticated informatic tools are necessary for the data mining process aimed at uncovering the differences in complex proteomic patterns [148]. By using these technologies, Petricoin and colleagues proposed a protein profile able to correctly identify 100% of 50 ovarian cancer cases, including 18 stage I patients, and to recognize 95% of 66 control cases as noncancerous [138].

Zhang and colleagues identified three potential biomarkers for ovarian cancer: apolipoprotein A_1 (downregulated in cancer); a truncated form of transthyretin (downregulated); and a cleavage fragment of inter-α-trypsin inhibitor heavy chain H4 (upregulated) [149]. In an independent validation to discriminate between stage I/II ovarian cancer cases and healthy controls, the multivariate model using the three biomarkers and CA 125, at a fixed sensitivity of 83%, had a significantly better specificity than CA 125 alone (94 vs 52%), with a cut-off corresponding to the same sensitivity of 83% (11 U/ml). On the other hand, CA 125 with a cut-off of 35 U/ml had a specificity of 97% and a sensitivity of 65%. At the same fixed specificity, the multivariate model using the three biomarkers and CA 125 had a sensitivity of 74%. However, the difference was not significant, partially due to the relatively few early stage ovarian cancer cases. The three identified biomarkers are highly present in serum, and two of them are cleavage products of precursor proteins by one or more proteases. These data further support the theory of an inbalance of protease and protease-inhibitor activity in the serum and tissue of cancer patients [60,150]. Moreover, transthyretin enhances the transport of retinol via its interaction with retinol-binding protein, and *in vitro* studies have suggested that decreased expression of retinol-binding protein and cellular retinol-binding protein 1 is associated with malignant transformation of the ovarian surface epithelium [149,151]. However, other authors advise great caution in considering transthyretin as a reliable biomarker for ovarian cancer, as the serum levels of this visceral protein are strongly affected by nutritional status,

inflammatory processes and hepatic diseases [152]. Therefore, the novel biomarkers identified from serum proteomic analysis should be further investigated and validated before being taken into consideration as components of an optimal panel of markers able to detect early stage ovarian cancer in the general population [149]. Recently, mass spectroscopic analysis of human serum showed that the majority of low-molecular-mass biomarkers are bound to carrier proteins such as albumin, thereby being protected from kidney clearance [153,154]. The analysis of selected albumin-bound protein fragments in pooled sera from women with early ovarian cancer, women with advanced ovarian cancer and healthy controls could offer a new biomarker archive for the detection of this malignancy. However, no significant clinical data are currently available.

Conclusions & future perspectives

Despite the controversies, serum tumor markers are important biochemical tools that can aid clinicians in the early detection of cancer, prediction of response to treatment, prognostic evaluation and monitoring the clinical course of disease [155]. The relevance of a serum tumor marker should be validated in a large, prospective study or a meta-analysis of small-scale retrospective/prospective studies before routine use [156]. Serum markers that have been validated at level 1 evidence include carcinoembryonic antigen (CEA) for the surveillance of patients with colorectal cancer, αFP, βHCG and lactate dehydrogenase (LDH) for evaluating the prognosis of patients with nonseminomatous germ-cell tumors, and CA 125 for monitoring therapy in patients with epithelial ovarian cancer. It is noteworthy that, in ovarian cancer, there are no approved blood tests for diagnosis and CA 125 is the only well validated tumor marker for use in monitoring disease course. A recent retrospective analysis showed that GCIG CA 125 response criteria are a better prognostic tool than RECIST for monitoring patients receiving topotecan or paclitaxel plus carboplatin as second-line treatment for recurrent disease [50]. However, CA 125 criteria and RECIST should be compared with different anticancer agents in randomized trials monitoring salvage chemotherapy. Several other tumor-associated antigens have been assessed as biomarkers for ovarian cancer, but their role is still under investigation. For instance, YKL-40 [54–56], hK6 [70,72], VEGF [104–110] and LPA [132,136] appear to be promising for diagnostic and prognostic purposes, but preliminary findings need validation in larger studies designed to yield precise estimates of the sensitivity and specificity of these novel biomarkers, both alone and in combination with other antigens (Table 5).

The future for tumor marker research is represented by the emerging technology of proteomics, which will probably allow scientific advances comparable to those achieved with the introduction of monoclonal antibody technology. However, the application of appropriate rules of evidence in the design, conduct and interpretation of clinical research on proteomic markers is strongly required [157].

Executive summary

Introduction

- The availability of ideal serum tumor markers would be of great clinical benefit for both the diagnosis and monitoring of epithelial ovarian cancer, which represents the leading cause of death from gynecologic cancer in Western countries.

Cancer antigen 125

- Cancer antigen (CA) 125 is the most reliable tumor marker for epithelial ovarian cancer.
- Serum CA 125 assay significantly increases the diagnostic reliability of ultrasound in discriminating a malignant from a benign ovarian mass, especially in postmenopausal women.
- Serum CA 125 kinetics during early chemotherapy correlates with response to treatment and survival of patients who receive platinum-based regimens or paclitaxel/platinum-based regimens.
- Changes in CA 125 levels correlate with regression, stability and progression of disease in 87–94% of instances and, moreover, rising antigen levels may precede the clinical detection of recurrence in 56–94% of cases, with a median lead time of 3–5 months.
- The Gynecologic Cancer Intergroup response criteria appear to be a better prognostic tool than the classical Response Evaluation Criteria in Solid Tumor in patients with recurrent disease treated with second-line topotecan or paclitaxel plus carboplatin.

Glycoprotein antigens other than CA 125

- CA 19.9, CA 15.3 and CA 72.4, first identified in gastrointestinal and breast cancers, have also been detected in the sera of patients with epithelial ovarian cancer.

Executive summary

- CA 19.9 is very sensitive for mucinous tumors, which often fail to express CA 125.
- Serum CA 125 correlates with disease course better than serum CA 19.9, CA 15.3 or CA 72.4.
- Elevated OVX1 values have been measured in a fraction of patients with normal CA 125 levels and persistent disease after first-line chemotherapy.
- The sensitivity of human cartilage glycoprotein-39 (YKL)-40 appears to be higher than that of CA 125 for early stage ovarian cancer.
- Elevated preoperative YKL-40 level appears to be an independent prognostic variable for survival.

Soluble cytokeratin fragments

- Serum cytokeratin-19 fragments in serum (CYFRA) 21–1 assay can discriminate a malignant from a benign ovarian mass, but it does not offer additional information to CA 125 assay alone.
- The prognostic relevance of serum CYFRA assay is uncertain.

Human-tissue kallikreins

- High serum levels of human kallikrein (hK) 6, 10 and 11 have been detected in more than 50% of patients with epithelial ovarian cancer, and these kallikreins are currently under evaluation as useful serum biomarkers for this malignancy.
- hK6 is slightly less sensitive, but more specific, than CA 125.
- Patients with high preoperative hK6 levels have a shorter progression-free survival and a overall survival.
- High serum hK10 is significantly related to advanced stage, serous histotype, high histologic grade, large residual disease, lack of response to chemotherapy and poor survival.

Cytokines

- Elevated serum levels of interleukin (IL)-6 and macrophage-colony stimulating factor (M-CSF) have often been detected in patients with epithelial ovarian cancer.
- The addition of serum IL-6 assay does not improve the sensitivity of CA 125 assay alone.
- An elevated preoperative IL-6 level is an independent poor prognostic factor for overall survival.
- Serum M-CSF appears to improve the diagnostic reliability of serum CA 125 alone.
- Serial M-CSF levels correlate significantly with disease course, but the concomitant determination of serum M-CSF adds little to serum CA 125 assay alone in the monitoring of disease course.

Vascular endothelial growth factor

- Vascular endothelial growth factor (VEGF) levels are markedly elevated in patients with advanced-stage or poorly-differentiated tumors or with large-volume ascites compared with those with early stage and well-differentiated tumors or with small-volume ascites.
- High pretreatment serum VEGF is an independent poor prognostic variable for survival.

Lysophosphatidic acid

- Elevated lysophosphatidic acid (LPA) levels have often been measured in blood samples from ovarian cancer patients, and LPA is currently under evaluation as a biomarker for this malignancy.
- In a recent study using a cut-off value of 1.5 µmol/, LPA assay has shown a sensitivity of 91.1% and specificity of 92.6% for epithelial ovarian cancer.
- Literature data on the role of LPA assay are conflicting, due to methodologic differences among different studies.

Proteomic markers

- Low-molecular-weight serum protein profiling might reflect the underlying pathologic state of an organ such as the ovary.
- In an early study, proteomic pattern analysis yielded a sensitivity of 100%, specificity of 95% and positive predictive value of 94% for epithelial ovarian cancer.
- Proteomic analysis has recently identified three potential biomarkers for ovarian cancer: apolipoprotein A_1 (downregulated in cancer), a truncated form of transthyretin (downregulated) and a cleavage fragment of inter-α-trypsin inhibitor heavy chain H4 (upregulated).
- The multivariate model using these three biomarkers and CA 125, at a fixed sensitivity of 83%, had a significantly better specificity than CA 125 alone (94 vs 52%), with a cut-off corresponding to the same sensitivity of 83% (11 U/ml). On the other hand, CA 125 with a cut-off of 35 U/ml had a specificity of 97% and sensitivity of 65%. At the same fixed specificity, the multivariate model using the three biomarkers and CA 125 had a sensitivity of 74%.
- The application of appropriate rules of evidence in the design, conduct and interpretation of clinical research on proteomic markers is strongly required.

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