

**Tumor markers**

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**INTRODUCTION**

A tumor marker is defined as a molecule or substance produced by or in response to neoplastic proliferation, which enters the circulation in detectable amounts. It indicates the likely presence of cancer or provides information about its behavior. Since the description of Bence-Jones proteins well over a century ago, a variety of substances have been investigated as potential tumor markers, and advances in molecular biology and technology continually add to this list.

Tumor markers can be broadly classified into tumor-specific antigens and tumor-associated antigens. Two examples of strictly tumor-specific antigens are the idiotypes of immunoglobulins of B cell tumors and certain neo-antigens of virus-induced tumors. The vast majority of tumor markers are in reality tumor-associated antigens. In many cases, they are initially described as highly tumor specific with subsequent studies uncovering their presence in multiple cancers and in normal adult or fetal tissues. On the basis of size, tumor-associated antigens can be divided into low-molecular weight tumor markers (approximately <1000 Daltons) and macromolecular tumor antigens. It is the macromolecular tumor markers that form the largest subgroup and have been most useful in the clinical management of cancer. A marker's performance depends on its sensitivity (proportion of cancers detected by a positive test) and specificity (proportion of those without cancer identified by a negative test), as well as the prevalence of the disease being tested in a particular population. An ideal tumor marker should have a 100% sensitivity, specificity, and positive predictive value. However, in practice such a marker does not exist. As the

majority of markers are tumor-associated rather than tumor-specific, and are elevated in multiple cancers, benign and physiological conditions, they lack specificity. In addition, varying sensitivity means that a normal result may not exclude malignancy. Hence, in most diseases, tumor markers contribute to differential diagnosis but are not themselves diagnostic. They may also have an important role to play in screening, surveillance, predicting prognosis, and determining therapeutic efficacy.

A wide variety of macromolecular tumor antigens, including enzymes, hormones, receptors, growth factors, biological response modifiers, and glycoconjugates have been investigated as potential tumor markers. Despite significant research, the number of clinically useful markers is limited. This is related to a variety of design issues both pre-analytical, such as selection bias and control matching, analytical, such as poor reproducibility, and post-analytical, such as statistical overfitting (Diamandis 2010, Jacobs and Menon 2011). As a result, they perform very differently when analyzed in an unbiased population based on prospectively collected samples (Zhu et al. 2011, Cramer et al. 2011, Timms et al. 2014). To remedy this, there is a push to adopt a standardized approach to biomarker studies, which includes separate roadmaps for biomarker development, depending on the application, and clinical validation where possible using a prospective specimen collection and retrospective blinded evaluation (PRoBE) design (Pepe et al. 2008). In addition, reporting recommendations, such as REMARK for tumor marker prognostic studies (Mcshane et al. 2005, Altman 2012) and STARD for tests of diagnostic accuracy (Korevaar et al. 2014, Bossuyt et al. 2003) have been proposed to overcome significant past reporting deficiencies in the published literature.

The focus of this chapter is largely limited to tumor markers that are detectable in the blood and are clinically relevant to female genital tract malignancies.

## **OVARIAN AND FALLOPIAN TUBE CANCER**

Ovarian cancer (OC) represents 1.7% of total incident cancers in women worldwide (Ferlay et al. 2012). The lifetime risk for developing OC is approximately 1% to 2%. Epithelial ovarian cancer (EOC) accounts for around 80% to 90% of all OC (Cancer Research UK 2011). EOC is a diverse group of tumors that can be classified based upon morphological and molecular features (Kurman and Shih 2010). Type I are thought to originate from borderline tumors and include low-grade serous, low-grade endometrioid, clear cell, mucinous, and transitional (Brenner) carcinomas. Type II include high-grade serous carcinoma (HGSC), undifferentiated carcinoma, and malignant mixed mesodermal tumors (carcinosarcoma). These are highly aggressive, evolve rapidly, and almost always present in advanced stage (Kurman and Shih 2010). HGSC is thought to originate from fallopian tube or cortical inclusion cysts related to fallopian tube epithelium (Nik et al. 2014). HGSC accounts for approximately 75% of OCs and over 90% of all deaths (Nik et al. 2014, Cho and Shih 2009). HGSC is more common in older women, with mucinous and endometrioid EOC, germ cell, and granulosa cell/sex-cord tumors more common in the reproductive age group.

### **Tumor Markers in Ovarian Cancer**

Only a few tumor markers have been validated for clinical use, with the best known among them being cancer antigen 125 (CA125). More recently, serum HE4 has been approved for use in EOC. The indications for use are detailed below.

#### ***CA125***

CA125 was first described by Bast in 1981. It is a 200-kd glycoprotein recognized by the OC125 murine monoclonal antibody (Bast et al. 1981). The CA125 structure includes two major antigenic domains: domain A (binds monoclonal antibody OC-125) and domain B (binds monoclonal antibody M11) (Nustad et al. 1996). The present second-generation heterologous CA125-II assay incorporates M11 and OC125 antibodies, while the original homologous assay was with OC125 alone. A number of CA125 assays which correlate well with each other are currently in clinical use (Davelaar et al. 1998).

CA125 is widely distributed in adult tissues and lacks specificity for OC. Although the exact cutoff might vary depending on the commercial assay, the cutoff is equivalent to the original cutoff of 35 U/mL, which is the 99th centile in a distribution of CA125 values in 888 healthy men and women (Bast et al. 1983). However, CA125 values can show wide variation, with lower levels (20 U/mL) found in postmenopausal women (Bon et al. 1996, Zurawski et al. 1988, Alagoz et al. 1994, Bonfrer et al. 1997). Levels are raised in pregnancy, with peak values occurring in the first trimester (112 U/mL, 65 U/mL correspond to 99th and the 96th centile, respectively) (El-Shawarby et al. 2005, Sarandakou et al. 2007) and postpartum (Spitzer and Kaushal 1998), and return to normal by 10 weeks after delivery (Spitzer and Kaushal 1998). Menstruation (Grover et al. 1992) as well as benign gynecological conditions (pelvic inflammatory disease, fibroids, and endometriosis) increase CA125. Higher values are reported for Caucasian compared to African or Asian women (Pauler et al. 2001). Caffeine intake, hysterectomy, and smoking in some (Pauler et al. 2001) but not all reports (Green et al. 1986) were associated with lower CA125 levels (Pauler et al. 2001). Non-gynecological conditions (tuberculosis,

cirrhosis, ascites, hepatitis, pancreatitis, peritonitis, pleuritis) and other cancers (breast, pancreas, lung, and colon cancer) can also cause an elevated CA125.

Raised levels were found in 25% of 59 stored serum samples collected 5 years before OC diagnosis (Zurawski et al. 1988), suggesting that CA125 is elevated in preclinical disease. An elevated CA125 (>35 U/mL) has been found in 85% of EOC (Zurawski et al. 1988, Canney et al. 1984), 50% Stage I, and >90% Stage II–IV cancer (Jacobs and Bast 1989). CA125 levels are more frequently elevated in serous cancers as compared to mucinous/borderline tumors (Jacobs and Bast 1989, Tamakoshi et al. 1996, Vergote and Bormer 1987).

#### ***Human Epididymis Protein 4***

Human epididymis protein 4 (HE4), is a glycoprotein found in epididymis epithelium. Increased gene expression of HE4 (WFCD2) and elevated serum levels have been reported in ovarian (Drapkin et al. 2005, Grisaru et al. 2007), as well as lung, breast, bladder, ureter transitional cell, pancreatic, and endometrial cancers (Huhtinen et al. 2009, Galgano et al. 2006). Serum HE4 levels are not increased in endometriosis (Huhtinen et al. 2009, Montagnana et al. 2009), and this results in fewer false positives compared to CA125 in differential diagnosis of adnexal masses (Heliström et al. 2003). HE4 levels appear to be lower in the Asian population (Park et al. 2012), and are decreased in pregnancy. The normal cutoff (95th centile) is 89 pmol/L and 128 pmol/L for premenopausal and postmenopausal women, respectively (Moore et al. 2012). Above the age of 40, serum HE4 concentrations rise, with dramatic changes seen in women above 55, leading to the recommendation that age-specific reference ranges be used (Urban et al. 2012). It has been reported to be elevated in over 50% of OC patients whose tumors do not express CA125 (Moore et al. 2008).

#### **Indications for Use of Tumor Markers**

## *Screening*

Serum CA125 continues to be investigated as a screening tool in clinical trials. In the ovarian screening arm of the Prostate Lung Colorectal and Ovarian Cancer Screening Trial (PLCO) 78,000 women were randomised to annual screening with TVS and CA125 (interpreted using a cut-off of  $\geq 35$  U/mL) or control groups. OC was diagnosed in 212 women (5.7 per 10000 person-years) of whom 118 died (3.1 per 10,000 person-years) in the intervention group. In the control group, 176 (4.7 per 10,000 person-years) women were diagnosed, of whom 100 died (2.6 per 10,000 person-years). There was no difference in mortality (RR, 1.18; 95% CI, 0.82–1.71) with screening. However, screening did result in an increase in invasive medical procedures with its associated risks. Of the 3285 women with false positive results, 1080 underwent surgical follow-up, of which 163 women experienced at least one serious complication (15%) (Buys 2011). This has resulted in reconfirmation that low-risk women should not be screened outside the context of clinical trials (US Preventative Services Task Force 2014).

More recently there has been a move in the context of screening to interpret serum CA125 levels using a more sophisticated approach, incorporating serial pattern of CA125 over time and age (Menon et al. 2005, Menon et al. 2009, Lu et al. 2013). This computerized algorithm, called the risk of ovarian cancer algorithm (ROCA) increases CA125 sensitivity by correctly identifying women with normal but rising levels while improving specificity by classifying women with static but elevated levels as low risk. In the general population UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) of 202,638 postmenopausal women, 50,639 women in the multimodal (MMS) arm underwent annual screening with CA125 interpreted using ROCA with second-line tests involving repeat CA125 and transvaginal

ultrasound (TVS). The performance characteristics of MMS were encouraging, with sensitivity, specificity, and positive predictive value for detection of primary ovarian/tubal cancers diagnosed within 1 year of screen being 89.4%, 99.8%, and 43.3% on the initial prevalence screen (Menon et al. 2009) and 82.8%, 99.8%, and 24.1%, respectively on incidence screening (Menon et al. 2015). The use of ROCA doubled the number of screen-detected invasive EOC detected during incidence screening compared to a fixed cutoff. Of the 155 women with invasive EOC, the ROCA detected 86.4%, whereas using annual serum CA-125 fixed cutoffs of >35, >30, and >22 U/mL would have identified only 41.3%, 48.4%, and 66.5%, respectively (Menon et al. 2015). This is in keeping with a retrospective analysis of the ovarian screening arm of the Prostate Lung Colorectal and Ovarian Cancer Screening Trial (PLCO) study. At 99% specificity, 20% of cases were identified on average 10 months earlier and at a lower CA125 concentration using a parametric empirical Bayes (PEB) longitudinal algorithm compared to a CA125 cutoff of  $\geq 35$  U/mL (Drescher et al. 2013). Recently the mortality results of UKCTOCS were published (Menon et al. 2015). There was a significant stage shift in primary invasive epithelial ovarian/tubal/peritoneal cancers on an intention-to-treat analysis in the multimodal arm compared to no screening (control) arm, with the proportion diagnosed in Stage I, II, IIIA 40% compared ( $p < 0.0001$ ) to 26% in the control group. The 15% average mortality reduction noted on primary analysis was not significant. It consisted of a reduction in mortality of 8% in years 0 to 7 from randomization and 23% for years 7 to 14. The delayed effect on mortality reduction was in keeping with other screening trials. OC-specific mortality was increasing at censorship in the control arm but seemed to have plateaued in the control arm. Additional follow-up is

now underway to confirm if there is a definitive mortality reduction (Jacobs et al. 2015).

In women at high risk for familial OC, annual screening with both CA125 and TVS has not been found to be effective (Hermsen et al. 2007). More frequent 3- to 4-monthly screening using the multimodal strategy was undertaken in UK Familial Ovarian Cancer Screening Study (UKFOCSS) Phase 2 as well as the Cancer Genetics Network (CGN) and Gynecological Oncology Group 0199 (Sherman et al. 2014) trials in the United States. While none of these trial results have been published, preliminary reports are available from conference proceedings. In the CGN trial, five OCs (following 38 surgeries) were detected in 2343 high-risk women undergoing 3-monthly screening. Four of these were early-stage cancers (Skates et al. 2007). In the larger UKFOCSS study of 4,348 women who underwent 13,728 women-years of screening, 19 cases were diagnosed, of which six were occult cancers found at risk reducing salpingoophorectomy (RRSO) and 13 were screen detected. There were no interval cancers. Fifty-two percent of the iEOC were Stage I/II (Rosenthal *et al.*, 2017). In BRCA mutation carriers the low incidence of primary peritoneal cancer following RRSO is similar to the general population and does not justify CA125 screening (Chen et al. 2014).

HE4 may have better sensitivity than TVS as a second-line screen (Urban et al. 2011).

Results from a small randomized controlled study of semiannual screening involving 208 high-risk women suggested that HE4 could be used as a confirmatory screening test following primary screening using CA125 (Karlán et al. 2014).

### **Differential Diagnosis and Prognosis**

Accurate discrimination between benign and malignant adnexal masses permits women with benign lesions to be managed conservatively or operated by general gynecologists while ensuring women with cancer are triaged to cancer centers for management by multidisciplinary teams and surgery by gynecological oncologists. CA125 using a cutoff of 35 U/mL had a pooled sensitivity and specificity of 78% for



differentiating benign from malignant adnexal masses, with higher values achieved in postmenopausal women (Myers et al. 2006).

A variety of modalities have been used to improve CA125 performance. The risk of malignancy index (RMI) is the oldest and most widely used. It is calculated by multiplying the serum CA125 level, an ultrasound based ovarian morphology score (U) and menopausal status (M) (Jacobs et al. 1990) and has a sensitivity of 85% and specificity of 97% (Jacobs et al. 1990) It has been validated in numerous prospective and retrospective studies (Andersen et al. 2003, Aslam et al. 2000, Bailey et al. 2006, Davies et al. 1993, Manjunath et al. 2001, Morgante et al. 1999, Tingulstad et al. 1996, Ulusoy et al. 2007).  $RMI \geq 200$  has been shown to be reliable in identifying patients who should undergo further preoperative imaging in a tertiary care setting (Håkansson et al. 2012). RMI sensitivity has been improved by increasing the RMI cutoff (Bailey et al. 2006, Davies et al. 1993) or modifying the RMI calculation (Manjunath et al. 2001). Recent modifications have included RMI IV which includes CA19-9 levels so as to better discriminate between borderline tumors and benign adnexal masses (Alanbay et al. 2012).

In a systemic review of women with suspected gynecologic disease, HE4 demonstrated a higher specificity (93% vs. 78%) and similar sensitivity (79%) to CA125 when distinguishing benign disease from OC (Ferraro et al. 2013). While HE4 and CA125 had similar diagnostic performance for EOC diagnosis, the former may be better at detection of borderline and early-stage cancers (Jacob et al. 2011) and in differentiation of EOC from ovarian metastases of gastrointestinal origin (Stiekema et al. 2015). Combining CA125 with HE4 was found to increase sensitivity while maintaining high specificity, and this has resulted in the development of the risk of ovarian malignancy algorithm (ROMA) (Moore et al. 2008). ROMA was shown to be

superior to CA125 alone in the differential diagnosis of a pelvic mass ,with an overall sensitivity of 93.8% at a specificity of 74.9% and a negative predictive value of 98% (Moore et al. 2009). In the subgroup of premenopausal women, it achieved a sensitivity of 100% (Moore 2011). A recent 2014 meta-analysis which included 32 studies evaluating the role of CA125, HE4, and ROMA concluded that HE4 performs better than CA125 and ROMA in the premenopausal population, with the reverse being true in the postmenopausal women (Wang et al. 2014). ROMA has been reported to have a higher receiver operating characteristics (ROC) area under the curve (AUC) for Type II EOC than Type I when compared to benign (Kristjansdottir et al. 2013).

OVA1 is a recently FDA-approved diagnostic test of the following five proteomic biomarkers: CA125, transthyretin (prealbumin), apolipoprotein A1,  $\beta$ 2 microglobulin, and transferrin. Recent studies from two groups have shown that OVA1 combined with physician assessment had higher sensitivity and net present value (NPV) than physician assessment alone and CA125 (Ueland et al. 2011, Bristow et al. 2013). These findings seemed to persist when only early-stage cancers were studied (Longoria et al. 2014). OVA1 has not been directly compared to ROMA. However, assimilated studies suggest that sensitivity and negative predictive value are likely to be similar but ROMA has greater specificity (73% vs. 43%) (Nolen and Lokshin 2013). A second-generation OVA1 (CA125, transferrin, apolipoprotein A-1, follicle-stimulating hormone, and HE4) has recently obtained FDA approval. This test provides significantly improved specificity (69% vs. 54%) and PPV (40% vs. 31%) compared to the first generation, while sensitivity (91% vs. 94%) and NPV (97% vs. 97%) remained unchanged. This improvement is of particular value as it decreases the number of benign masses requiring gynecological oncology referral (Coleman et al.

2015).

ADNEX is a recently proposed model that uses CA125, age, and type of gynecology center as well as six ultrasound predictors. It has the ability to discriminate between five types of adnexal tumors (benign, borderline, Stage I cancer, Stage II–IV cancer, and secondary metastatic cancer) and also between benign and all malignant tumors. The model's ability to discriminate between advanced primary and secondary metastatic cancer is attributed to the CA125 level. ADNEX's performance seems to be similar to or better than logistic regression model 2 (LR2) and simpler (Van Calster et al. 2014), but needs external validation.

Controversy persists on the most appropriate test for differential diagnosis of adnexal masses. Results comparing ROMA versus RMI are conflicting (Moore 2010, Van Gorp et al. 2012). In a recent meta-analysis which evaluated 19 prediction models in 96 validation studies, ultrasound models, simple rules and the LR2 outperformed RMI in diagnostic accuracy, particularly in premenopausal women (Kaijser et al. 2014). A key limitation is that almost all studies were based on women who underwent surgery for adnexal masses, and therefore performance characteristics cannot be accurately extrapolated to include women with adnexal lesions that were managed conservatively.

### **Prognosis**

A detailed literature review of epidemiological studies undertaken up to 2009 concluded that serum CA125 is a strong prognostic factor for OC. Levels are inversely related to progression-free and overall survival. Levels following surgery and during the first three cycles of chemotherapy together with CA125 half-life and nadir have been found to be independent prognostic indicators (Gupta and Lis 2009). This extends to levels in the normal range with pre-maintenance chemotherapy

patients with baseline CA125 values  $\leq 10$  U/mL or  $\leq 5$  U/mL having greater progression-free survival compared to those with higher “normal” levels (van Altena et al. 2010, Markman et al. 2006). A gradual, as opposed to abrupt, rise seems to be associated with longer progression-free and overall survival (Levy et al. 2013).

There is great interest in trying to predict complete cytoreduction. A preoperative CA125 level  $>500$  has been associated with a high risk of suboptimal cytoreduction (Suidan et al. 2014). Other studies have suggested that HE4 (Angioli et al. 2013) or a combination of HE4 and CA125 might be better predictors of surgical outcome (Braicu et al. 2013). A CA125 level of  $<75$  U/mL after the third cycle of neo-adjuvant chemotherapy has recently been reported to independently predict complete cytoreduction at interval debulking surgery (Pelissier et al. 2014).

### **Monitoring Response to Treatment and Recurrence**

Serial serum CA125 forms part of most standard protocols for evaluating response to treatment (Söletormos et al. 2012), although not an integral part of the RECIST (v1.1) criteria (Eisenhauer et al. 2009). Levels correlate with clinical course of EOC and may also be of benefit in women with  $\alpha$ -fetoprotein (AFP) and human chorionic gonadotropin (hCG)-negative germ cell tumors (Patterson and Rustin 2006). There is emerging data that HE4 may detect recurrence earlier than CA125 and additionally have a role to play in women whose tumors do not express CA125 (Schummer et al. 2012).

Various CA125-based definitions for recurrence have been suggested, such 2- to 2.5-fold increase from baseline (Tuxen et al. 2001, Rustin et al. 2001) with an interval of 3 to 4 months (range 1–15 months) between increase and clinical detection of progressive disease (Tuxen et al. 2001, Cruickshank et al. 1991). It is important to highlight that the randomized controlled trial OV05 showed no survival benefit on

commencing treatment on the basis of rising CA125 levels in the absence of other indicators of disease recurrence (Rustin et al. 2010).

### **Carcinoembryonic Antigen**

Carcinoembryonic antigen (CEA) is a 180-kDa glycoprotein, initially described as a tumor marker for gastrointestinal, prostate, and lung cancers (Icard et al. 1994).

Raised levels have been reported in endometrioid, mucinous, and Brenner tumors (Sólétermos et al. 1996). It is often used to differentiate between EOC and ovarian metastases of gastrointestinal origin (Stiekema et al. 2015). An added benefit is that it is not elevated in benign and inflammatory adnexal masses, and pregnancy and does not differ with menopausal status (Kondalsamy-Chennakesavan et al. 2013).

### **CA-19-9**

CA-19-9 is an monosialoganglioside secreted by mucinous tumors of the gastrointestinal tract, including the pancreas and biliary tree (Pavai and Yap 2003). It is more frequently elevated in mucinous (76%) than serous (27%) EOC (Gocze et al. 1988, Terracciano et al. 2005). Markedly elevated serum levels (>1000 U/mL) may be found in benign mucinous neoplasms as well as in borderline and malignant tumors. Elevated levels cannot be used to predict whether an ovarian mucinous tumor is benign, borderline, or malignant (Kelly et al. 2010).

### **Alpha-Fetoprotein and Human Chorionic Gonadotropin**

AFP (70-kDa glycoprotein) is synthesized initially in the yolk sac and subsequently in the fetal liver and intestine (Gitlin et al. 1972). AFP is increased in pregnancy, benign liver disease, and liver, gastric, pancreatic, colon, and bronchogenic malignancies. Elevated levels are seen in most endodermal sinus/yolk sac ovarian tumors and correlate with the extent of the disease. It is useful in monitoring treatment response and in assessing early recurrences (Parkinson et al. 2011). With regard to other germ

cell tumors, increased AFP levels have been reported in 33% to 62% of immature teratomas and 12% of dysgerminoma and embryonal tumors (Lu 2005, Kawai et al. 1992). Beta human chorionic gonadotropin ( $\beta$ hCG) levels are universally raised in the rare ovarian choriocarcinoma and in 5% of patients with dysgerminoma. Mixed germ cell tumor can secrete either, both, or none, depending on the components. In patients with dysgerminoma, an AFP or  $\beta$ hCG level of  $>100$  IU/L indicates the presence of nondysgerminomatous elements (Berek and Hacker 2005). Tumor markers are negative in pure immature teratomas.

### **Inhibin and Anti-Mullerian Hormone**

Inhibin is a heterodimeric glycoprotein with two isoforms: inhibin-A and inhibin-B. Serum inhibin is elevated in ovarian granulosa cell/sex cord/stromal tumors and has a useful role in differential diagnosis and surveillance of these malignancies (Lappohn et al. 1989, Boggess et al. 1997, Geerts et al. 2009). Granulosa cell tumors secrete both inhibin-A and inhibin-B, though the latter is more common (Petraglia et al. 1998). Inhibin is considered more reliable and superior to estradiol E2 in monitoring and predicting recurrence in granulosa cell tumors (Pectasides et al. 2008). AMH is a dimeric glycoprotein, a member of the transforming growth factor- $\beta$  family, and is produced by the granulosa cell (La Marca et al. 2010). It is more specific for granulosa cell tumors than inhibin, as inhibin may also increase in some (mucinous) epithelial ovarian tumors (Burger et al. 1996). In patients with elevated inhibin-B and/or AMH levels at initial diagnosis, it can be used during follow-up. Currently, there is no evidence-based preference for inhibin-B or AMH as a tumor marker (Geerts et al. 2009).

### **Future Tumor Markers**

Many published biomarkers are not validated in independent studies, leading to a paucity of clinical useful tests. However, there are some promising potential tumor markers on the horizon. The most exciting are tumor-derived cell-free nucleic acids (DNA and RNA) (Kamat et al. 2010) and circulating tumor cells (Romero-Laorden et al. 2014, Ma et al. 2014).

Circulating tumor DNA (ctDNA) is usually detected by sequencing for tumor specific mutations. In HGSC, TP53 mutation are ubiquitous (Ahmed et al. 2010). Forshew et al. (2012) have recently reported detection of high levels of ctDNA using tagged-amplicon deep sequencing (TAm-Seq) for TP53 mutations in 2% to 65% in plasma from patients with advanced OC. Further optimization is required, together with an increase in sensitivity, but this technique does offer the potential of a noninvasive, low-cost, and high-throughput “liquid biopsy.” Autoantibodies to tumor-derived proteins, p53, PTPRA, and PTGFR as potential biomarkers for early detection of OC are being investigated (Anderson et al. 2015).

There is also a move to explore OC biomarkers in novel samples. Studies have detected tumor DNA from OC in liquid-base cervical cytology specimens (Kinde et al. 2013). Recent reports suggest sensitivity for detection of OC can be improved by lavage of the the endometrial cavity using a three-way catheter to obtain samples that can then be tested for somatic mutations using massively parallel sequencing (next-generation sequencing) (Maritschnegg et al. 2015).

## **CERVICAL CANCER**

In the UK, approximately 3100 cases and 1000 deaths from cervical cancer occur annually (CRUK 2014). Around two-thirds of cases are squamous cell carcinoma and 15% are adenocarcinoma. Seventy-eight percent of cases are diagnosed in 25- to 64-year-olds, with peak incidence occurring in the 30- to 34-year age group (CRUK

2014). Cervical cancer screening using cytology or HPV DNA testing of cervical specimens is one of the most successful public health interventions in the developed world, and serological tumor markers do not currently play a role. However, a variety of serum markers have been investigated in assessing prognosis, monitoring response to treatment, and detecting recurrence.

### **Squamous-Cell Carcinoma Antigen**

Squamous-cell carcinoma (SCC) antigen (Kato 1977) has two isoforms: SCC1 (neutral isoform) and SCC2 (acidic isoform). Elevated serum levels are more common in women with well (78%) and moderately (67%) differentiated carcinoma than in poorly differentiated tumors (38%) (Crombach et al. 1989), confirming that it is a marker for squamous cell differentiation. Squamous and adenosquamous tumors are more likely to have elevated levels than pure adenocarcinomas (Kawaguchi et al. 2013). SCC levels correlate positively with lymph node metastasis, although a normal level cannot exclude it (Takeda et al. 2002, Yoon et al. 2007).

Serum SCC levels reflect response to treatment, and rising levels often precede recurrence (Gadducci et al. 2007). In a recent study, response to chemotherapy was more accurately predicted by SCC than by MRI, with a combination of the two further improving predictive power (Yin et al. 2013). Persistently elevated or rising post-treatment SCC levels are indicative of disease persistence or progression.

### **Other Tumor Markers**

CEA has low sensitivity (38%) but high specificity (98%) for cervical adenocarcinoma (Ngan et al. 1996, Borrás et al. 1995), and levels are reported to correspond with extent of disease (Yoon et al. 2007, Molina et al. 2005). Serum CA125 levels are raised in 20% to 75% of women with cervical adenocarcinoma, and may be useful for monitoring patients (Gadducci et al. 2007). CYFRA 21-1 has been



found to be elevated in 34% to 63% of cervical cancer, with higher values reported in adenocarcinoma and late-stage disease (Pras et al. 2002, Piao et al. 2015).

### **GESTATIONAL TROPHOBLASTIC TUMORS/NEOPLASIA**

Gestational trophoblastic disease (GTD) comprises a wide spectrum of disorders, with gestational trophoblastic tumors (GTT) or gestational trophoblastic neoplasia (GTN) representing the malignant end of the spectrum (Ngan and Seckl 2007). The UK incidence is 1 in 387 live births in Asian women and 1 in 752 live births in non-Asian women (Tham et al. 2003). Although GTN may occur after any pregnancy, it is 2000 times more common following a molar pregnancy. Malignant transformation occurs in 16% complete and 0.5% partial molar pregnancies (Ngan and Seckl 2007). Accurate diagnosis is paramount, as it is almost always possible to cure GTN and preserve fertility. Survival rates of 100% and 95% have been obtained for low-risk and high-risk disease, respectively (Froeling and Seckl 2014).

### **Human Chorionic Gonadotropin**

Human chorionic gonadotropin (hCG) is an oncofetal antigen (glycoprotein), which consists of two subunits ( $\alpha$  and  $\beta$ ) and is normally secreted by the syncytiotrophoblast. While the  $\beta$ -subunit is distinct and responsible for biologic and immunologic specificity, the  $\alpha$ -subunit is common to other anterior pituitary hormones. It has a half-life of 24 to 48 hours, though this is much shorter for the individual subunits. In normal pregnancy,  $\beta$ hCG is largely intact and is only hyperglycosylated in the first trimester. However, in trophoblastic disease or cancer, the  $\beta$ hCG can exist in a number of fragments, including nicked hCG,  $\beta$ -core, C-terminal segment, and free  $\beta$ -subunit. Hence in cancer it is important to use hCG assays that detect all forms of  $\beta$ hCG. An inability to detect some hCG variants may lead to false negative test results, and miss detection of active disease or recurrence. Some assays may lead to

an increase in false positive results due to cross-reacting heterophile antibodies (Froeling & Seckl 2014). No commercial assay is licensed for use in cancer diagnosis at present. In the UK, a non-commercial rabbit polyclonal antibody is used for assay. Seckl et al. (2010) have recommended use of the Siemens IMMULITE (Deerfield, IL, USA) as the only commercial assay that seems comparably safe to use.

$\beta$ hCG is an “ideal tumor marker” for GTN, as its levels are universally raised and correlate with tumor burden and therapeutic response. It is very sensitive for small-volume disease, and plays a primary role in the management of GTN. The diagnosis of GTN is dependent on failure of  $\beta$ hCG serum levels to regress following either a normal or abnormal pregnancy. In addition to diagnosis, hCG is an integral part of FIGO staging and risk scoring systems, and is used for monitoring treatment and detecting recurrence (Agarwal et al. 2012, Seckl et al. 2013, Seckl et al. 2010).

Placental site trophoblastic tumors (PSTT) produce low levels of  $\beta$ hCG, and undetectable serum levels do not equate to lack of tumor. Presence of  $\beta$ -core fragment in the urine may aid in the diagnosis of PSTT. In PSTT, serum  $\beta$ hCG is not thought to be helpful in predicting survival (Schmid et al. 2009).

## **ENDOMETRIAL CANCER**

Around 93% of endometrial cancers occur in postmenopausal women. In the reproductive age group it is mainly linked to genetic predisposition (e.g., Lynch syndrome), obesity, or polycystic ovary syndrome. With prolonged life expectancy and rising obesity, the incidence is expected to rise. None of the serum markers have a well-established role in the clinical management of endometrial cancer.

Serum CA125 is elevated in 10% to 34% of patients. Elevated preoperative levels have been found to correlate with advanced stage (Powell et al. 2005), higher grade, increased depth of myometrial invasion, positive peritoneal cytology, and nodal

involvement (Chen et al. 2011, Jiang et al. 2015). CA125 levels of >35 U/mL and >105 U/mL in women aged >49 and ≤49 years, respectively, have been reported to be associated with poor survival (Chao et al. 2013). It maybe useful in follow-up but evidence is limited (Otsuka et al. 2010, Kurihara et al. 1998, Lo et al. 1997).

Recent studies suggest that preoperative HE4 is better correlated to myometrial invasion and primary tumor diameter than CA125 (Brennan et al. 2014, Kalogera et al. 2012). This maybe useful for preoperative risk stratification, allowing identification of patients who may benefit from lymphadenectomy at surgical staging.

### **VULVAR AND VAGINAL CANCER**

Tumors of the vulva and the vagina are uncommon, and only a few studies have described circulating markers in these cancers, which include tissue polypeptide–specific antigen, SCC, and urinary gonadotropin fragment (Salman et al. 1995, Carter et al. 1995, Nam et al. 1990). There is currently no role for serological markers in the clinical management of these cancers.

### **SUMMARY**

CA125 remains the most widely investigated and clinically used tumor marker for epithelial OC. As part of the RMI and more recently ADNEX, it plays a crucial role in differential diagnosis of adnexal masses. Newer tests include ROMA, which combines HE4 with CA125, and OVA1, where a further panel of markers has been added to the duo. Serial CA125 measurements are used to monitor treatment and detect recurrence. However, no survival benefit has been reported on commencing treatment on the basis of rising CA125 levels in the absence of clinical recurrence. Multimodal screening using serial CA125 and second-line TVS has been shown in trial to have encouraging performance characteristics for detecting invasive OC. However, it is not currently recommended outside the trial setting, as the mortality

impact of screening is not yet available. The most promising tumor markers for future clinical use include tumor-derived DNA and circulating tumor cells. AFP and  $\beta$ hCG are routinely used in germ cell tumors and inhibin and AMH in ovarian granulosa cell/sex cord/stromal tumors.

$\beta$ hCG is the “ideal tumor marker” for GTN and is integral to diagnosis, staging, and monitoring therapeutic response. Using hCG assays that detect all forms of  $\beta$ hCG is essential in cancer patients to minimize false negative results.

SCC is the commonest tumor marker used for cervical carcinoma. Serum SCC levels in squamous cell carcinoma reflect response to treatment, and rising levels often precede recurrence. Serological markers are currently not used in the routine clinical management of the other gynecological malignancies.

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