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Protein biomarkers of ovarian cancer: the forest and the trees

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Abstract

The goal of effective population-based screening for ovarian cancer remains elusive despite intense efforts aimed at improving upon biomarker and imaging modalities. While dozens of potential serum biomarkers for ovarian cancer have been identified in recent years, none have yet overcome the limitations that have hindered the clinical use of CA-125. Avenues of opportunity in biomarker development are emerging as investigators are beginning to appreciate the significance of remote, as well as local or regional, sources of biomarkers in the construction of diagnostic panels, as well as the importance of evaluating biomarkers in prediagnostic settings. As the list of candidate biomarkers of ovarian cancer continues to grow, refinements in the methods through which specific proteins are selected for further development as components of diagnostic panels are desperately sought. Such refinements must take into account both the bioinformatic and biological significance of each candidate. Approaches incorporating these considerations may potentially overcome the challenges to early detection posed by the histological heterogeneity of ovarian cancer. Here, we review the recent progress achieved in efforts to develop diagnostic biomarker panels for ovarian cancer and discuss the challenges that remain.

Keywords

biomarkers; host response; ovarian cancer; pelvic mass; screening; tumor microenvironment

Ovarian cancer represents the eighth most common cancer among women and the second most frequently diagnosed gynecological malignancy in the USA and Europe [1]. The overall mortality attributed to ovarian cancer exceeds that of any other gynecological cancer with over 50% of the 200,000 women or more newly diagnosed each year worldwide

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expected to perish from the disease [201]. A critical factor in the elevated mortality associated with ovarian cancer is the lack of disease-specific symptoms. This difficulty is underscored in a joint recommendation recently issued by a high-profile consortium of public health organizations termed the Ovarian Cancer Symptom Index, which listed bloating, pelvic or abdominal pain, difficulty eating/fullness and urinary symptoms as those more likely to occur in ovarian cancer patients than healthy women [2]. Compounding the problem of ubiquitous clinical presentation is the observation that the majority of early-stage cancers are asymptomatic, resulting in over three-quarters of all diagnoses being made at a time when the disease has already established regional or distant metastases [3]. Despite aggressive cytoreductive surgery and platinum-based chemotherapy, the 5-year survival rate for patients with clinically advanced ovarian cancer is only 15–20%, although the cure rate for stage I disease is usually greater than 90% [3,4]. This strongly suggests that finding and removing tumors that remain confined to the ovary should confer a substantial improvement in survival. A stochastic model developed for annual screening indicated that shifting diagnosis from late (stage III) to early (stage I) stages could result in 3.4 years of life saved per patient [5]. Therefore, biomarker-based screening methodologies aimed at detecting ovarian cancer at its earliest stages have the potential to result in substantial improvements in overall survival for this disease.

Screening for early disease: requirements & reality

Considering the low prevalence of ovarian cancer, any proposed screening strategy must demonstrate a minimum specificity (SP) of 99.6% and a sensitivity (SN) of >75% to achieve a positive predictive value of 10% and avoid an unacceptable level of false-positive results [6,7]. Present methods for detecting ovarian cancer include physical examination, assessment of symptoms, imaging by several modalities (transvaginal ultrasound [TVS], Doppler ultrasound, CT, MRI and PET), and serum levels of the CA-125 biomarker of ovarian cancer [8–10]. CA-125 is the most robust and well-known serum biomarker for detection of ovarian cancer. The initial finding of CA-125 levels greater than 35 U/ml in approximately 83% of patients with advanced epithelial ovarian cancer and in only 1–2% of the normal population led to investigations into its use as a biomarker for ovarian cancer [11,12]. Furthermore, elevated levels of CA-125 are most strongly associated with serous tumors, which represent the most aggressive histological type of ovarian cancer [13]. Subsequent analyses of CA-125 have revealed a number of limitations for the test. Although CA-125 is frequently elevated in advanced-stage ovarian cancer, the protein is elevated in less than 50% of stage I disease and is often normal in mucinous, endometrioid and clear-cell carcinomas [14–20]. Moreover, a number of benign and malignant conditions may result in falsely elevated CA-125 values [21,22]. Additional factors may influence the CA-125 level, such as race/ethnicity, age, hysterectomy, smoking history and obesity [23]. Despite these well-recognized limitations, CA-125 is the most widely studied serum biomarker for ovarian cancer. The best currently available protocol for early detection of ovarian cancer, a combination of screening for elevated CA-125 and TVS in the presence of elevated CA-125 [24,25], does not meet the stringent criteria for cost–effectiveness espoused by the US Preventive Services Task Force [26]. As a result, no professional group currently recommends screening for ovarian cancer in the general population [27–29]. Thus, there is a critical need to develop additional informative biomarkers capable of complementing CA-125 in order to achieve the requisite diagnostic performance necessary for clinical advancement.

Two large, randomized trials that were designed to determine whether ovarian-cancer screening improves survival have completed the enrollment of patients. In the Prostate, Lung, Colon, and Ovarian (PLCO) Cancer Screening Trial, 34,261 healthy women between the ages of 55 and 74 years of age were randomly assigned to undergo either annual CA-125

testing plus TVS or to receive 'usual care' [30]. A positive finding was defined as a CA-125 level of more than 35 U/ml or TVS evidence of an abnormal ovarian volume, or an ovarian cyst with papillary projections or solid components. Diagnostic follow-up of positive screens was performed at the discretion of participants' physicians. The positive predictive value of a positive screening test was 1.0–1.3% during the 4 years of screening. The overall ratio of surgeries to screen-detected cancers was 19.5:1. Importantly, 72% of screen-detected cases were stage III or IV, indicating that screening has not resulted in stage shift [30]. The PLCO project team recently released its report in which they conclude that the CA-125/TVS screening approach does not reduce disease-specific mortality in comparison with usual care, but does result in an increase in invasive medical procedures and associated harms [31].

In the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), 202,638 postmenopausal women between the ages of 50 and 74 years, who were deemed to be at average risk for ovarian cancer, were randomly assigned to undergo annual pelvic examination (control group), annual TVS (ultrasonography or USS group) or annual measurement of CA-125 (evaluated over time with the use of ROCA) plus TVS in cases in which the CA-125 level was elevated (multimodality or MMS group) [32]. Women with persistent abnormality on repeat screens underwent evaluation by a clinical oncologist and, where appropriate, surgery. As compared with ultrasonography alone, multimodality screening had a significantly greater SP (99.8 vs 98.2%) and a higher positive predictive value (35.1 vs 2.8%); ($p < 0.001$); SN did not differ significantly between the two groups. The proportion of primary invasive ovarian and fallopian tube cancers diagnosed with stage I/II disease (48%) was encouraging compared with the 26% rate in the clinical series [33] and 22% in the prevalence screen of the PLCO screening trial in the USA [34]. Both the USS and MMS arms demonstrated a higher proportion of stage I–II cancers. The apparent difference between PLCO and UKCTOCS in proportion of screen-detected early cases could possibly be explained by the mode of diagnostic follow-up utilized in each trial wherein the suspicious cases identified in the PLCO trial were cared for by their physicians and in the UKCTOCS trial the majority were referred to a gynecologic oncologist.

Ovarian cancer screening biomarkers: the forest

Phases of cancer biomarker development

Biomarker development efforts to date clearly indicate that no individual biomarker, including CA-125, can provide sufficient SN at high SP for the early detection of ovarian cancer. Therefore, in order to devise a robust multimarker algorithm, it is necessary to identify additional informative biomarkers that complement CA-125. According to the requirements of the Early Detection Research Network/NIH, biomarker development should include five phases; preclinical exploratory Phase I, clinical development and validation in Phase II, validation in a retrospective longitudinal study in Phase III, prospective screening in Phase IV and randomized clinical trials in Phase V [35].

Methodological approaches to protein biomarkers development

Noninvasive biomarker testing is essential for practical general population monitoring. Therefore, biomarkers that can be detected in bodily fluids such as blood, urine, saliva or cervical mucus could be considered. The candidate marker must be both stable in these body fluids and present in quantities that can quantitatively differentiate individuals with ovarian cancer from individuals without disease. In the arena of Phase I protein biomarker development, a number of conceptual and methodological approaches have been utilized for biomarker discovery in bodily fluids. Early efforts, including those responsible for the identification of CA-125, described the analysis of murine antibodies capable of reacting

with tumor cells from ovarian cancer patients [36–38]. Later, biomarker discovery was achieved primarily by means of high-throughput screening methodologies, such as genomic, transcriptional and proteomic profiling [39–50]. Proteomic profiling provides direct information about proteins differentially expressed in cancer patients and control individuals. Such profiling has been performed by mass spectrometry [40] and, lately, through the use of antibody arrays, such as multiplex bead-based arrays or reverse antigen arrays [51,52]. Both approaches have limitations. To date, MS-based methods have demonstrated limited analytical SN and, consequently, most of the biomarkers discovered by proteomics represent high abundance, predominantly acute-phase proteins [53]. Recently, developed methods aimed at the depletion of abundant serum proteins may overcome this limitation and lead to the enhanced discovery of lower-abundance biomarkers [54,55]. Immunoassays permit the analysis of proteins present in bodily fluids at much lower concentrations – as low as fg/ml – but this approach is limited by the availability of suitable immunoassays. However, the menu of candidate biomarker immunoassays continues to expand as the popularity of multiplex bead-based platforms grows. For instance, three large screening exercises aimed at the identification of ovarian cancer biomarkers using multiplex bead-based immunoassays evaluated over 300 candidate biomarkers in serum obtained from large groups of ovarian cancer patients and controls [51,52,56].

Individual biomarkers of ovarian cancer: the trees

Our review of the relevant literature identified over 160 proteins reported to be differentially expressed in early ovarian cancer compared with healthy controls (Table 1). The ongoing analysis of these proteins, and the biological and pathological pathways they regulate could provide powerful tools for determining the mechanisms of ovarian cancer initiation and progression that could eventually lead to individualized biological therapies. However, as mentioned above, further validation steps are necessary to determine the value if these proteins, and relatively few have been evaluated in studies that meet the criteria for Phases II and/or III biomarker development [51,52,57–61].

Origin of ovarian cancer biomarkers: tumor–stroma interaction

The complex and multifaceted process of ovarian epithelial tumorigenesis necessitates the development of an equally complex tumor microenvironment. Within this microenvironment, tumor cells, stromal fibroblasts and infiltrating leukocytes produce a multitude of autocrine and paracrine factors, including but not limited to cytokines, chemokines, growth factors, hormones, inflammatory mediators and acute-phase reaction molecules. It is this complex signaling network that mediates tumor/stroma interactions and facilitates tumor proliferation, invasion, neoangiogenesis and metastasis. As illustrated in Table 1, concentrations of numerous proteins involved in the above listed tumorigenic biological functions listed above are altered in the circulation of ovarian cancer patients. Mediators of inflammation, including cytokines and acute-phase reactants, were the most numerous among the various biological groups identified in our review, followed by adhesion molecules/proteases, growth/angiogenic factors and hormones.

Biomarkers of ovarian cancer reflect different biological pathways of ovarian tumorigenesis

The Ingenuity® pathway analysis software was utilized in an in-depth functional analysis of the ovarian cancer biomarkers presented in Table 1. This analysis identified acute-phase response signaling as a top canonical pathway and inflammatory response as a top biological function associated with most ovarian cancer biomarkers (97/157 biomarkers = 62%). In addition, cellular movement, cell-to-cell signaling, proliferation and cell death were identified as primary molecular and cellular functions associated with ovarian cancer

biomarkers (Table 2). With respect to network analysis, most ovarian cancer biomarkers fall into three major networks responsible for key aspects of cancer development (Table 3). Network 1 (70/157 = 45% of molecules) regulates invasion/metastasis, neoangiogenesis and immune responses; network 2 (24% of molecules) is responsible for DNA functions; and network 3 (14% of molecules) controls the cell cycle and inflammation. Of these multiple biomarkers, demonstrating strong association with ovarian cancer, the biomarkers with the highest informative value, singly or combined into multibiomarker panels, must be selected. In this selection process (feature selection), a two-pronged approach must be utilized considering both bioinformatics and the biological aspects of each biomarker or biomarker combination.

Feature selection

Biological considerations

The selection of the most informative biomarkers represents the most crucial and challenging aspect of biomarker development. Prior to applying a bioinformatics-based feature selection, candidate biomarkers must be prioritized based on biological considerations that should include the following characteristics:

- A biomarker should demonstrate measurable alterations in noninvasive samples obtained from patients with early-stage or preclinical disease;
- Collectively, biomarkers should be informative for all histological and pathological subtypes of a given cancer. In the setting of ovarian cancer, this includes the histological types (e.g., serous, mucinous, endometrioid, clear cell and less common subtypes), and the pathologically distinct type I and II tumors (described in [62]);
- Individual biomarkers should be sufficiently reliable. The justification for a sufficiently high intraclass correlation index between temporal replicates depends on two factors: the magnitude of the intraclass correlation; and the degree of overlap, or difference in mean expression, for the distributions of that biomarker (i.e., between cases and controls). Specifically, the 95% confidence interval for the median difference between cases and controls should exclude 0 for a given biomarker to be considered in further analysis.

Bioinformatics approaches

Biomarkers prioritized based on biological considerations should be further filtered using bioinformatic approaches that rely on optimized feature selection of high-dimensional data. The rule of thumb for feature selection is that features must be discriminant and add information to existing biomarkers in the panel for the best separation of the two groups. In the context of ovarian (or any other) cancer, the additive information feature means that selected biomarkers should increase SN at the same high SP over CA-125 used alone. Feature selection algorithms can be divided into three categories: filters, wrappers or embedded methods [63]. A filter method ranks features based on an evaluation measure, and then selects a subset of features that are ranked highly (e.g., two sample t-test). Using this method, biomarkers were selected from Phase II to Phase III in a PLCO collaborative exercise [64]. A wrapper method searches for the best set of features by repeated selection and assessment of a subset of features using a classification algorithm. An example of a wrapper method is the approach utilized in our work based on a Metropolis algorithm with Monte Carlo simulation [51]. Embedded methods include feature selection inside of the classifier training process. For example, decision trees implicitly determine which features are more discriminant and use them in the learning of tree structures. Finally, the resulting combination should not only be sensitive and specific but also selective for a given

condition with respect to other nonrelated diseases that have the potential to confound clinical diagnosis. Below, we will discuss some aspects of feature selection.

Approaches & controversies in biologically based feature selection

Tumor versus stroma as sources of biomarker origin

There are several conceptual beliefs regarding biomarker prioritization that were not included in the list above. For example, there is an opinion that only those proteins that are directly released by tumor cells should be considered as cancer biomarkers [65]. In accordance with this notion, many of the biomarkers listed in Table 1 would not be considered as they are known to originate from various extratumoral sources (e.g., liver for acute-phase proteins and pituitary gland for pituitary hormones). However, growing evidence suggests that many of these so-called extratumoral biomarkers are likely to be secreted by the growing tumor. For example, ovarian tumors and primary fallopian cells *in vitro* are capable of secreting, in addition to CA-125, HE4 and CA 15–3, multiple acute-phase reactants, inflammatory mediators, and additional molecules [Nolen LBM, Lokshin AE, Unpublished Data] [66–68]. For example, the gastrointestinal hormone, ghrelin, has been shown to be expressed in the human ovary and also in inclusion cysts within the ovarian surface epithelium, where it induces cell proliferation, indicating a potential tumorigenic role [69]. Likewise, matrix metalloproteinases (MMPs) could originate from multiple sources within the tumor microenvironment, including tumor cells themselves, as well as the stroma, where they are presumably produced in response to growth factor and cytokine signaling [70,71]. Another example is the pituitary hormone, prolactin, which has been shown to be produced by several nonpituitary sources, including ovarian tumor cells, using an alternate promoter; and also functions as an ovarian tumor promoter *in vitro* [72]. It is possible that proteins secreted from tumor and from extratumoral sources are differentially modified. For example, previous reports have indicated alterations in haptoglobin, α1-acid glycoprotein and α1-antichymotrypsin in ovarian cancer patients [73]. Ovarian tumor cells also secrete cytokines that can influence glycan processing in both the tumor cells and surrounding tissue [74]. Recent data indicate that ovarian tumor cells secrete factors that alter the glycosylation profile of liver proteins, and raise the possibility that some of the altered glycoforms of haptoglobin, α1-acid glycoprotein and α1-antichymotrypsin may be tumor derived [75].

Acute-phase reactants

All biological aspects of feature selection are empirical by default, meaning that every conclusion should be experimentally supported. One example illustrating the necessity of experimental confirmation is the recent debate on the value of acute-phase reactants as ovarian cancer biomarkers for early detection. The acute-phase is controlled by the balance of proinflammatory cytokines (IL-6, IL-8, IL-1b, TNF-α and IFN-γ), anti-inflammatory molecules (IL-4, IL-10, IL-13 and TGF-β) and the inhibitors of proinflammatory cytokines (soluble TNF-α receptor, soluble IL-1 receptor and IL-1). Proinflammatory cytokines stimulate the increased production of positive acute-phase proteins (C-reactive protein [CRP], serum amyloid A, haptoglobin, α1-acid glycoprotein, α1-antitrypsin, α1-antichymotrypsin and fibrinogen) and decreased production of negative acute-phase proteins (albumin and transferrin) by the liver [76,77]. As shown above, multiple ovarian cancer biomarkers represent acute-phase reactants and acute-phase response signaling is a top canonical pathway of ovarian cancer. Several statements have been published casting doubt on the usability of acute-phase reactants for early detection of ovarian cancer based on the belief that these biomarkers are altered as a part of an inflammatory response to late-stage ovarian cancer [78–81]. However, acute-phase reactants, as well as many proteins conventionally considered to represent late systemic responses could, in fact, play a

causative role in cancer development. For example, inflammatory cytokines and acute-phase response proteins could be a part of an initial 'para-inflammation' phenomenon provoked by a premalignant lesion [82]. During a para-inflammatory response, leukocytes and other phagocytic cells produce an array of cytokines and reactive nitrogen and oxygen species, that upon repeated exposure, result in permanent DNA damage in endothelial and epithelial cells [83–85]. Additionally, cytokines and acute-phase reactants play multiple roles in early cancer development, from inducing tumor growth and inhibiting apoptosis to stimulating angiogenesis (Table 1) [85–92]. For example, apart from being a serine protease inhibitor, α1-antitrypsin stimulates the production of HGF, and was found to induce the proliferation of fibroblasts and the synthesis of procollagen in culture [93,94]. Another acute-phase reactant, ceruloplasmin, which serves as the major copper transport enzyme in blood, also acts as a molecular switch for activating proangiogenic factors [95]. The potential differential expression of acute-phase reactants/inflammatory molecules in early-stage or premalignant lesions has to be ascertained experimentally. In fact, levels of the acute-phase reactant, B2M, were altered in the sera of women participating in the PLCO trial who were diagnosed with ovarian cancer 0–2 years after the blood draw [64,96].

Upregulated versus downregulated cancer biomarkers

Another paradigmatic concept of a diagnostic biomarker holds that a particular protein must be increased in biological fluids from cancer patients. The findings presented in Tables 1–4 indicate the need for a revision of this concept as proteins that are present at lower circulating levels in cases versus controls have been incorporated into useful biomarker panels. Examples of this type of biomarker include EGF receptor (EGFR) and ApoA1, two biomarkers selected based on their usefulness in diagnostic panels which are observed at lower levels in ovarian cancer patients in comparison with controls and are likely to be produced, at least in part, by extratumoral local or systemic sources [51,52,57,58,97,98]. Further review of the findings reported in several expansive biomarker studies which utilized multiplex bead-based immunoassay platforms [Nolen LBM, Lokshin AE, Unpublished Data] [51,99] identified a total of 111 biomarkers found to be significantly altered between the case and control groups (Table 5). By consensus, 69 or 62% of the significant biomarkers found in these studies were elevated in the ovarian cancer groups, while 41 (or 38%) were decreased. With the exception of carbohydrate antigen tumor markers (CA-125, CA 19–9, CA 15–3 and CA 72–4), which are all elevated in the cancer groups, each other functional category presented in Table 1 is represented by both overexpressed and underexpressed biomarkers. Some protein families contain both up- and downregulated representatives, such as the IGF family of proteins, where IGFBP-1, -2, -4 are increased in ovarian cancer while IGF-1 and IGFBP-3, -6 are decreased (Table 5). The differential pattern of serum alterations reported in our reviewed studies suggests a complex mechanism for the various member of the IGF family in ovarian cancer. Similarly, among MMPs, MMP-2 and -3 were observed to be downregulated in ovarian cancer while MMP-7 and -9 were elevated. The majority of the hormones examined in the three studies were found to be downregulated in ovarian cancer. Interestingly, these underexpressed hormones include FSH, whose serum levels normally increased in post-menopausal women [100,101]. It has been speculated that this decrease indicates increased binding of FSH to its receptors, which is irreversible at body temperature and results in permanent removal from the circulation [102]. Other protein biomarkers underexpressed in ovarian cancer include ApoA1 and transthyretin (TTR), which have been evaluated extensively as components of diagnostic panels for ovarian cancer and may originate from tumoral, local or systemic sources [62,68,103,104]. The adhesion molecule, VCAM-1, was observed to be downregulated in ovarian cancer in two studies and this alteration led to its inclusion in a highly effective diagnostic panel in our recent report [51]. Among other biomarkers downregulated in the circulation of patients with ovarian cancer are eotaxin-1 and -2, IL-18,

macrophage-derived chemokine, XCL1, EGFR, BDNF, ErbB2, IGFBP-3 and -6, IGF-I, PEDF, SCF, VCAM-1, MMP-2 and -3, NCAM, adiponectin, ERα, ghrelin, GIP, insulin, leptin, leuteinizing hormone, osteocalcin, thrombopoietin, α2-macroglobulin, ApoA1, CIII, H, clusterin, complement C3 and C4, factor VII, fetuin A, TTR, Fas, perforin and CK-MB (Table 4). While mechanisms leading to lower circulating concentrations of these proteins are likely to vary, some may be attributed to increased clearance from the circulation through renal filtration. This notion is supported by the observation of increased urinary concentrations of eotaxin-1, EGFR, ErbB2 and VCAM-1 in patients with ovarian cancer as compared with healthy individuals [Nolen LBM, Lokshin AE, Unpublished Data]. Elsewhere, irreversible binding to cognate receptors on proliferating tumor cells [105] or internalization could result in lower blood levels. Additionally, differential glycosylation patterns could affect half-life of some biomarkers in circulation [75,106,107].

Biomarkers of different subtypes of ovarian carcinoma

Histological subtypes

Current evidence strongly suggests differential mechanisms of pathogenesis, genetic alterations and clinical behavior among the major histological subtypes of ovarian cancer, which include serous, endometrioid, mucinous and clear cell carcinomas (reviewed in [108]). These subtypes are increasingly considered to be distinct diseases originating from separate nonovarian tissues through mechanisms involving the presence or absence of specific precursors, including endometriosis. The tumor marker CA-125 is most highly associated with high-grade serous carcinomas, the most prevalent and aggressive ovarian cancer subtype, with other subtypes exhibiting only limited expression [18–20].

A number of the most intensely evaluated ovarian cancer biomarkers, including several of those selected in diagnostic panel development (Table 4) have shown some evidence of subtype SP in various reports. Expression of carcinoembryonic antigen (CEA) is associated with the mucinous subtype [109], and CEA was consistently selected by our MMC algorithm as part of a multimarker panel for a set of ovarian cancers representing different histologies [Nolen LBM, Lokshin AE, Unpublished Data] [51]. The expression of MMP-2, -7 and -9 have been well described in serous ovarian carcinomas with links to disease progression [1,110,111]. Expression of MMP-7 and -14 has also been noted in mucinous and clear cell carcinomas, respectively [112,113]. Expression of IGF-II by ovarian cancer cells was found to be associated with serous histology and this expression was driven by distinct promoters in comparison with nonserous histologies [114,115]. In separate studies, VEGF was found to be frequently expressed in both serous and clear cell ovarian carcinomas, and was proposed as a potential therapeutic target for these subtypes [2,116]. M-CSF is expressed by both serous and mucinous ovarian cancers, and is believed to stimulate the phenotypic shift of tumor-associated macrophages to M2, thus promoting tumor growth [117]. Based on our reviewed findings and another recent publications, it would appear that high-grade serous carcinoma is associated with a larger array of aberrantly expressed biomarkers than other subtypes [118]. When biomarker combinations were considered, the panel of ApoAI, TTR and TF demonstrated particular utility in the detection of mucinous and endometrioid ovarian carcinomas [68,104,119].

Classes of ovarian cancer: type I versus type II

Kurman and colleagues have described two classes of ovarian cancer. Type I tumors are low-grade serous, low-grade endometrioid, clear cell, mucinous, and transitional carcinomas that exhibit Ras and Raf mutations, slow indolent growth and generally present as early stage. Type II tumors include high-grade serous, undifferentiated and malignant mixed mesodermal cancers with p53 mutations, are highly aggressive, evolve rapidly and present at

an advanced stage [62]. Currently, the most urgent unmet need is to more effectively screen for type II cancers, where the clinical benefit will be greatest. This will require sensitive and specific biomarkers that are expressed during ovarian carcinogenesis and reach the peripheral circulation at levels distinguishable from those in healthy individuals at a point sufficiently early to permit interventions that can alter the natural history of the disease. If, however, detecting type II ovarian cancer at an early stage proves difficult, the identification of women with small volume disease offers a reasonable alternative, as these are patients known to benefit most from chemotherapy. Detection of type II cancers from within high-risk populations has been particularly difficult and the use of multiple markers might improve SN [120]. To date, only one study examined levels of multiple biomarkers (MIF, Prolactin, CA-125, OPN, leptin, IGF-II and autoantibodies to p53) in plasma of type I and type II ovarian cancer and concluded that these biomarkers had significantly different plasma levels between type II ovarian cancer patients and healthy controls [121].

Selection of multimarker panels based on postdiagnostic samples

A number of studies evaluating multimarker panels in postdiagnostic samples utilizing various criteria for biomarker prioritization and selection were reviewed (Table 4). In the study by Mor *et al.*, 169 proteins were analyzed in serum samples obtained from ovarian cancer patients by means of rolling circle amplification (RCA) immunoassay microarray, and 35 of these proteins were found to be differentially expressed between cases and controls [56]. Biomarkers were further prioritized for independent ELISA testing if similar levels of expression were observed in newly diagnosed patients and patients with recurrent disease; ELISA tests were available commercially for these analytes; and they had compelling biological reasons for their evaluation. These analytes were EGF, MIF-1, TNF-α, leptin, prolactin, IL-17, OPN and IGF-II. Leptin, prolactin, OPN and IGF-II showed perfect correlation between the RCA microarray immunoassays and the ELISA assays, and were able, when used together, to discriminate the control and cancer training group samples with 95% SN at 95% SP. Adding CA-125 and MIF to this panel provided a classification power of 92% SN at 99% SP. This panel was equally sensitive to different histologic subtypes of primary epithelial ovarian cancers – papillary serous, clear cell, endometroid and mixed [60]. Unfortunately, following a high level of initial enthusiasm and the subsequent marketing of this panel under the trade name OvaSure™ (Labcorp, NC, USA), deficiencies in study design have been identified that illustrate the challenges facing biomarker development efforts in general. Most prominent among these deficiencies was the inaccurate calculation of positive predictive value based on improper estimates of ovarian cancer prevalence [122,123]. This observation, coupled with the lack of evaluation in a large prospective study, led to performance revisions and the withdrawal of the commercial kit.

In the recent study by Havrilevsky *et al.* [124], a literature review was conducted for investigations describing transcriptional profiling of epithelial ovarian cancer. Candidate biomarkers meeting the following inclusion criteria were selected: overexpression of candidate gene in epithelial ovarian cancer relative to normal ovarian epithelium; overexpression of encoded protein in ovarian tissue; localization of encoded proteins to extracellular compartment as membrane-bound or secreted protein; and discrimination of ovarian cancer from normal sera utilizing prototype immunological assays. Correlation with CA-125 was not evaluated. Based on these criteria, eight genes – *HE4*, glycodelin (*PAEP*), *MMP-7*, *MUC-1*, *PAI-1*, *SLPI*, *Plau-R* and *inhibin A* – were selected as candidate markers for inclusion. Several combinations of these proteins were evaluated and a combination of CA-125, HE4, glycodelin, Plau-R and MMP-7 was selected, which offered 79% SN at 93% SP for early-stage ovarian cancer [124].

Our group utilized a subject cohort that included more than 2000 healthy women in an unbiased analysis of serum biomarker candidates [51]. Feature selection was performed based on both bioinformatics and the following biological considerations: high accuracy in early-stage disease; equal classification of all ovarian cancer histologies; and selectivity for ovarian cancer versus benign disease and other epithelial cancers [51]. Our identified panel of CA-125, HE4, CEA and VCAM-1 was found to discriminate early-stage ovarian cancer from the control group with 86% SN at 98% SP.

In a multicenter case–control study, Zhang *et al.* utilized SELDI-TOF to analyze sera obtained from 195 patients with invasive epithelial or other types of ovarian cancer along with patients diagnosed with benign pelvic masses and healthy control women [61]. The investigators employed subsets of five separate centers for sequential components of their analysis. Two centers were designated for biomarker discovery and candidate peaks from the proteomic analysis were identified by cross-validation. These candidate biomarker peaks were then validated in sera collected at two additional sites. Validated biomarker peaks were then identified and the corresponding proteins purified. Candidate biomarkers for which suitable immunoassays were available were then chosen for validation in sera collected at the fifth site. Analysis of immunoassay results led to the identification of a four-biomarker panel consisting of CA-125, ApoA1, TTR and H418. In the validation set, this panel provided a SN of 74% at a SP of 97% for the discrimination of early-stage ovarian cancer from healthy women.

In a separate study, Skates *et al.* evaluated serum levels of four biomarkers by immunoassay in patients enrolled at three independent collection centers [59]. Sera collected at the first center constituted the training set and biomarker data were analyzed using three statistical models: logistic regression, classification tree and mixture discriminant analysis (MDA). The MDA model is unique in that it emulates the heterogeneity of ovarian cancer with respect to histological subtype and utilizes the distribution of biomarker levels within each subtype to estimate the histological type of each sample analyzed. The authors conclude that logistic regression and MDA modeling perform similarly and both outperform classification tree in the discrimination of ovarian cancer cases from controls. The combination of CA-125, CA 15–3, CA 72–4 and M-CSF, identified using the MDA model, performed best in the independent validation set, providing a SN of 70% at 98% SP for early-stage ovarian cancer.

Recently, Edgell *et al.* reported on a retrospective case–control study (Phase II biomarker study) in which the authors utilized a multiplexed bead-based immunoassay platform to evaluate five ovarian cancer biomarkers (CA-125, CRP, SAA, IL-6 and IL-8) in 362 plasma samples obtained from ovarian cancer patients and healthy controls split into independent training and validation sets [58]. Through multivariate modeling, the authors demonstrated a SN and SP of 94.1 and 91.3%, respectively, for the five-biomarker panel in the validation set and a SN and SP of 92.3 and 91.3%, respectively, for all early-stage ovarian cancers.

In another recent study, Amonkar *et al.* demonstrated the value of multiplexed analysis in their evaluation of 104 candidate biomarkers in a cohort of over 350 ovarian cancer patients and controls [57]. The cohort represented various stages of disease, and each of the most common histological subtypes of epithelial ovarian cancer and most prevalent benign ovarian conditions. Their training analysis led to the identification of an 11-analyte profile consisting of CA-125, CA 19–9, EGFR, CRP, myoglobin, ApoA1, ApoCIII, MIP-1α, IL-6, IL-18 and tenascin C. In an independent validation set, this panel provided a SN of 91.3% at a SP of 88.5%.

Multimarker panels in prediagnostic samples

While many of the reports represented in Table 4 are initially encouraging, several hurdles remain before any of the identified models can be implemented clinically on a widespread basis. Foremost is the need to evaluate the most promising panels in prospective randomized clinical trials. Additional preclinical validation will be required to fully characterize the efficacy of selected panels before this significant next step is warranted. A key component of this validation process is the evaluation of panels in samples obtained from prediagnostic ovarian cancer patients. Progress towards this type of validation is greatly hindered by the rarity of this sample type; however, several significant findings have been reported by a group under the direction of Nicole Urban. In a pair of reports, this group first describes elevated levels of CA-125, HE4 and mesothelin in the sera of symptomatic ovarian cancer patients, and then in the sera of patients 0–3 years prior to diagnosis, noting an optimal lead time of 1 year [125,126]. In a separate study, the investigators utilize a combinatorial approach, including CA-125 and HE4 measurements, in addition to the Symptom Index to prospectively classify benign from malignant pelvic masses with a SN of 84% at a SP of 98.5% (Table 4) [127]. These findings regarding the usefulness of CA-125 and HE4 in prediagnostic and prospective samples are especially encouraging given the attention this combination has received in recent reports.

Recently, a collaborative study was performed to assess the prediagnostic performance of candidate biomarkers in the PLCO trial [64,128]. Forty one biomarkers selected by five leading ovarian cancer biomarker laboratories from Harvard University, Fred Hutchinson Medical Center, MD Anderson Cancer Center University of Pittsburgh and Yale University were utilized in the analysis of 118 cases and 473 matched healthy controls [64,96]. The selection of these markers proceeded from a Phase II validation study using a common reference set of 160 ovarian cancer cases (~50% stage I/II) and 480 general population controls based solely on individual performance (SN at 95% SP, AUC). The study demonstrated that none of these biomarkers or multimarker panels added any appreciable improvement in SN over that of CA-125 alone. Our subsequent analyses revealed that ten of the selected biomarkers: HE4, mesothelin, CA 15–3, CA 72–4, KLK6, spondin-2, TTR, Cyfra 21–1, IGFBP2 and YKL-40, were strongly ($r > 0.35$) correlated with CA-125 [Nolen LBM, Lokshin AE, Unpublished_Data]. This lack of feature independence could be credited as a limiting factor in the ability of these additional biomarkers to complement the diagnostic performance of CA-125.

In nearly all cases, efforts aimed at novel biomarker test development have relied upon the use of biological fluid samples collected at the time of first diagnosis. It would appear from the disappointing results of the PLCO study and others that this approach is not optimized for the development of prediagnostic tests offering a potential window of early successful intervention. Ongoing efforts of this type are unlikely to be productive, and the incorporation of new strategies focused on the characterization and detection of preclinical disease will be necessary for true advancement. Recent work suggests that a precursor lesion for serous carcinomas, termed serous tubal intraepithelial carcinoma (STIC), does indeed exist [129]. STICs originate from the fimbriated end of the fallopian tube, indicating a tubal, rather than ovarian epithelial, origin for serous carcinomas. The identification of the potential STIC precursor of serous carcinomas represents an important finding that may lead to advances in detecting these tumors at an earlier stage. Perhaps analysis of blood samples from patients who had undergone prophylactic oopherectomy where preneoplastic lesions were discovered could prove useful, although substantial effort would be needed to collect a statistically meaningful set of such samples. Finally, prospective identification of preclinical biomarkers using mouse models of ovarian cancer could be helpful.

Conclusion

The search for biomarker-based screening tools for the early detection of ovarian cancer has yielded a number of promising findings. Biomarker panels offering levels of SN and SP exceeding 90% have been identified by a number of groups utilizing a variety of analysis techniques and statistical models. Each of the panels identified demonstrates a clear performance advantage over the individual performance of CA-125. Clinical implementation of biomarker tools has been delayed, and sometimes reversed, in large part owing to the stringent performance requirements associated with the detection of a rare disease and the lack of a demonstrated survival benefit.

The rapidly expanding appreciation of the heterogeneity of ovarian cancer presents biomarker researchers with a unique set of questions and challenges. Among these questions, the leading question is whether early detection efforts should target each of the disease subtypes collectively or focus on the most troublesome high-grade serous tumors. Clinical evidence describing the indolent nature of type I tumors (low-grade serous, endometroid, mucinous, clear cell), which are readily detected by current practices suggest the latter strategy might be most prudent. The difficulty in detecting the rapidly progressing high-grade serous tumors at an early manageable stage also indicates a need to focus biomarker development efforts on that individual subtype. However, within the setting of differential diagnosis of a pelvic mass, an ideal test would be capable of identifying tumors of multiple histological subtypes prior to referral for invasive diagnostic procedures. A biomarker panel consisting solely of conventional tumor markers (i.e., proteins derived directly from tumor cells and elevated in bodily fluids) is unlikely to accomplish this given the divergent molecular characteristics displayed by the various ovarian cancer subtypes. Likewise, although preferentially expressed by high-grade serous tumors, CA-125 alone is not sufficient for diagnosis or screening. The development of truly useful and clinically applicable biomarker-based diagnostics will require a broad and meticulous search through the forest of ovarian cancer biomarkers. This search should be conducted with an eye for biological relevance and bioinformatic performance, and should employ flexible criteria that permit the consideration of a diverse set of candidates.

The limited performance of serum biomarkers in the testing of prediagnostic samples indicates the need to identify additional useful biomarkers that may display alterations earlier in disease development. Further investigation into the tumorigenic pathways associated with each subtype of epithelial ovarian cancer is likely to provide a basis for additional biomarker discovery and development. The identification of the potential STIC precursor of serous carcinomas represents an important finding that may lead to advances in detecting these tumors at an earlier stage. Currently, findings related to the subtype-specific production of ovarian cancer biomarkers are sporadic and incomplete. The ongoing paradigm shift regarding the tissue of origin and pathogenesis of disease subtypes should give rise to additional investigations of this type. A greater appreciation of the natural history of each subtype of ovarian cancer, including the involvement or absence of precursor lesions, should further refine the search for useful prediagnostic biomarkers.

Future perspective

Biomarker-based screening tools for ovarian cancer are likely to advance well into clinical practice over the next 5–10 years. Implementation in the setting of routine screening will depend largely upon continually emerging findings from prospective clinical trials along the lines of PLCO and UKCTOCS. Physicians, researchers and public health officials will be required to weigh the potential survival benefits against the economic and social tolls associated with population-based screening. The continued improvement and refinement of

screening tools, including the identification of biomarker panels providing diagnostic capabilities superior to that of CA-125, should steadily tip the balance in favor of implementation. The incorporation of extratumoral biomarkers and the use of alternative analytical biofluids, such as urine, will likely contribute to this advancement. A paradigm shift in our definition of ovarian cancer biomarkers will likely be coupled to this advancement in biomarker-based tools. The increased identification and utilization of proteins derived from extratumoral biological compartments, proteins observed at decreased levels in patients relative to healthy individuals and proteins that provide complementary value through SP for a particular disease subtype is likely to characterize progress related to biomarker research.

References

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Yoshida H, Ishiko O, Sumi T, Matsumoto Y, Ogita S. Survivin, bcl-2 and matrix metalloproteinase-2 enhance progression of clear cell- and serous-type ovarian carcinomas. Int J Oncol. 2001; 19(3):537–542. [PubMed: 11494033]
2. Brustmann H, Naude S. Vascular endothelial growth factor expression in serous ovarian carcinoma: relationship with high mitotic activity and high FIGO stage. Gynecol Oncol. 2002; 84(1):47–52. [PubMed: 11748975]
3. Holschneider CH, Berek JS. Ovarian cancer: epidemiology, biology, and prognostic factors. Semin Surg Oncol. 2000; 19(1):3–10. [PubMed: 10883018]
4. Baker TR, Piver MS. Etiology, biology, and epidemiology of ovarian cancer. Semin Surg Oncol. 1994; 10(4):242–248. [PubMed: 8091065]
5. Skates SJ, Singer DE. Quantifying the potential benefit of CA 125 screening for ovarian cancer. J Clin Epidemiol. 1991; 44(4–5):365–380. [PubMed: 2010780]
6. Jacobs IJ, Menon U. Progress and challenges in screening for early detection of ovarian cancer. Mol Cell Proteomics. 2004; 3(4):355–366. [PubMed: 14764655]
7. Menon U, Jacobs IJ. Ovarian cancer screening in the general population: current status. Int J Gynecol Cancer. 2001; 11(Suppl 1):3–6. [PubMed: 11488995]
8. Bast RC Jr, Xu FJ, Yu YH, Barnhill S, Zhang Z, Mills GB. CA 125: the past and the future. Int J Biol Markers. 1998; 13(4):179–187. [PubMed: 10228898]
9. Kinkel K, Hricak H, Lu Y, Tsuda K, Filly RA. US characterization of ovarian masses: a meta-analysis. Radiology. 2000; 217(3):803–811. [PubMed: 11110947]
10. Fishman DA, Cohen L, Blank SV, et al. The role of ultrasound evaluation in the detection of early-stage epithelial ovarian cancer. Am J Obstet Gynecol. 2005; 192(4):1214–1221. discussion 1221–1212. [PubMed: 15846205]
11. Zurawski VR Jr, Orjaseter H, Andersen A, Jellum E. Elevated serum CA 125 levels prior to diagnosis of ovarian neoplasia: relevance for early detection of ovarian cancer. Int J Cancer. 1988; 42(5):677–680. [PubMed: 3182103]
12. Bast RC Jr, Klug TL, St John E, et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med. 1983; 309(15):883–887. [PubMed: 6310399]
13. Tseng PC, Sprance HE, Carcangiu ML, Chambers JT, Schwartz PE. CA 125, NB/70K, and lipid-associated sialic acid in monitoring uterine papillary serous carcinoma. Obstet Gynecol. 1989; 74(3 Pt 1):384–387. [PubMed: 2761916]
14. Jacobs I, Bast RC Jr. The CA 125 tumour-associated antigen: a review of the literature. Hum Reprod. 1989; 4(1):1–12. [PubMed: 2651469]

15. Woolas RP, Xu FJ, Jacobs IJ, et al. Elevation of multiple serum markers in patients with stage I ovarian cancer. J Natl Cancer Inst. 1993; 85(21):1748–1751. [PubMed: 8411259]

16. Slotman BJ, Rao BR. Ovarian cancer (review) Etiology, diagnosis, prognosis, surgery, radiotherapy, chemotherapy and endocrine therapy. Anticancer Res. 1988; 8(3):417–434. [PubMed: 3291746]

17. Yancik R. Ovarian cancer. Age contrasts in incidence, histology, disease stage at diagnosis, and mortality. Cancer. 1993; 71(Suppl 2):517–523. [PubMed: 8420671]

18. Gocze P, Vahrson H. Ovarian carcinoma antigen (CA 125) and ovarian cancer (clinical follow-up and prognostic studies). Orv Hetil. 1993; 134(17):915–918. [PubMed: 8479736]

19. Molina R, Filella X, Jo J, Agusti C, Ballesta AM. CA 125 in biological fluids. Int J Biol Markers. 1998; 13(4):224–230. [PubMed: 10228906]

20. Kaku T, Ogawa S, Kawano Y, et al. Histological classification of ovarian cancer. Med Electron Microsc. 2003; 36(1):9–17. [PubMed: 12658347]

21. Neesham D. Ovarian cancer screening. Aust Fam Physician. 2007; 36(3):126–128. [PubMed: 17339973]

22. Clarke-Pearson DL. Clinical practice Screening for ovarian cancer. N Engl J Med. 2009; 361(2): 170–177. [PubMed: 19587342]

23. Johnson CC, Kessel B, Riley TL, 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(3):383–389. [PubMed: 18586313]

24. Menon U. Ovarian cancer screening. CMAJ. 2004; 171(4):323–324. [PubMed: 15313987]

25. Urban N, McIntosh MW, Andersen M, Karlan BY. Ovarian cancer screening. Hematol Oncol Clin North Am. 2003; 17(4):989–1005. ix. [PubMed: 12959188]

26. Calogne N. Screening for ovarian cancer: recommendation statement. Ann Fam Med. 2004; 2(3): 260–262. [PubMed: 15209204]

27. US Preventive Services Task Force. Screening for ovarian cancer: recommendation statement. Am Fam Physician. 2005; 71(4):759–762. [PubMed: 15756773]

28. ACOG committee opinion. Role of loop electrosurgical excision procedure in the evaluation of abnormal Pap test results Number 195, November 1997 Committee on Gynecologic Practice American College of Obstetricians and Gynecologists. Int J Gynaecol Obstet. 1998; 61(2):203–204. [PubMed: 9639230]

29. Brown DL, Andreotti RF, Lee SI, et al. ACR appropriateness criteria(c) ovarian cancer screening. Ultrasound Q. 2010; 26(4):219–223. [PubMed: 21084936]

30. Partridge E, Kreimer AR, Greenlee RT, et al. Results from four rounds of ovarian cancer screening in a randomized trial. Obstet Gynecol. 2009; 113(4):775–782. [PubMed: 19305319]

31▪▪. Buys SS, Partridge E, Black A, et al. Effect of screening on ovarian cancer mortality: the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Randomized Controlled Trial. JAMA. 2011; 305(22):2295–2303. The Prostate, Lung, Colon and Ovarian trial report on the impact of CA-125/transvaginal ultrasound multimodal screening on ovarian cancer mortality. [PubMed: 21642681]

32▪▪. Menon U, Gentry-Maharaj A, Hallett R, et al. Sensitivity and specificity of multimodal and ultrasound screening for ovarian cancer, and stage distribution of detected cancers: results of the prevalence screen of the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). Lancet Oncol. 2009; 10(4):327–340. Preliminary results of United Kingdom Collaborative Trial of Ovarian Cancer Screening prospective trial. [PubMed: 19282241]

33. Ueland FR, Depriest PD, Pavlik EJ, Kryscio RJ, Van Nagell JR Jr. Preoperative differentiation of malignant from benign ovarian tumors: the efficacy of morphology indexing and Doppler flow sonography. Gynecol Oncol. 2003; 91(1):46–50. [PubMed: 14529661]

34. Buys SS, Partridge E, Greene MH, et al. Ovarian cancer screening in the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial: findings from the initial screen of a randomized trial. Am J Obstet Gynecol. 2005; 193(5):1630–1639. [PubMed: 16260202]

35. Pepe MS, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001; 93(14):1054–1061. [PubMed: 11459866]

36. Koprowski H, Herlyn M, Steplewski Z, Sears HF. Specific antigen in serum of patients with colon carcinoma. Science. 1981; 212(4490):53–55. [PubMed: 6163212]
37. Gold P, Freedman SO. Demonstration of tumor-specific antigens in human colonic carcinomata by immunological tolerance and absorption techniques. J Exp Med. 1965; 121:439–462. [PubMed: 14270243]
38. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981; 68(5):1331–1337. [PubMed: 7028788]
39. Husseinzadeh N. Status of tumor markers in epithelial ovarian cancer has there been any progress? A review. Gynecol Oncol. 2011; 120(1):152–157. [PubMed: 20934205]
40. Zhang B, Barekati Z, Kohler C, et al. Proteomics and biomarkers for ovarian cancer diagnosis. Ann Clin Lab Sci. 2010; 40(3):218–225. [PubMed: 20689132]
41. Makridakis M, Vlahou A. Secretome proteomics for discovery of cancer biomarkers. J Proteomics. 2010; 73(12):2291–2305. [PubMed: 20637910]
42. Dutta S, Wang FQ, Phalen A, Fishman DA. Biomarkers for ovarian cancer detection and therapy. Cancer Biol Ther. 2010; 9(9):668–677. [PubMed: 20372062]
43. Tung CS, Wong KK, Mok SC. Biomarker discovery in ovarian cancer. Womens Health (Lond Engl). 2008; 4:27–40. [PubMed: 19072449]
44. Coticchia CM, Yang J, Moses MA. Ovarian cancer biomarkers: current options and future promise. J Natl Compr Canc Netw. 2008; 6(8):795–802. [PubMed: 18926090]
45. Mclemore MR, Miaskowski C, Aouizerat BE, Chen LM, Dodd M. Rules of tumor cell development and their application to biomarkers for ovarian cancer. Oncol Nurs Forum. 2008; 35(3):403–409. [PubMed: 18467290]
46. Merritt WM, Sood AK. Markers of angiogenesis in ovarian cancer. Dis Markers. 2007; 23(5–6): 419–431. [PubMed: 18057525]
47. Badgwell D, Bast RC Jr. Early detection of ovarian cancer. Dis Markers. 2007; 23(5–6):397–410. [PubMed: 18057523]
48. Munkarah A, Chatterjee M, Tainsky MA. Update on ovarian cancer screening. Curr Opin Obstet Gynecol. 2007; 19(1):22–26. [PubMed: 17218847]
49. Bast RC Jr, Badgwell D, Lu Z, et al. New tumor markers: CA125 and beyond. Int J Gynecol Cancer. 2005; 15(Suppl 3):274–281. [PubMed: 16343244]
50. Nossov V, Amneus M, Su F, et al. The early detection of ovarian cancer: from traditional methods to proteomics Can we really do better than serum CA-125? Am J Obstet Gynecol. 2008; 199(3): 215–223. [PubMed: 18468571]
51▪▪. Yurkovetsky Z, Skates S, Lomakin A, et al. Development of a multimarker assay for early detection of ovarian cancer. J Clin Oncol. 2010; 28(13):2159–2166. Identification of a four-biomarker panel with high performance in the discrimination of ovarian cancer from a large group of healthy control women. [PubMed: 20368574]
52. Bertenshaw GP, Yip P, Seshaiah P, et al. Multianalyte profiling of serum antigens and autoimmune and infectious disease molecules to identify biomarkers dysregulated in epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2008; 17(10):2872–2881. [PubMed: 18843033]
53. Moshkovskii SA, Serebryakova MV, Kuteykin-Teplyakov KB, et al. Ovarian cancer marker of 11.7 kDa detected by proteomics is a serum amyloid A1. Proteomics. 2005; 5(14):3790–3797. [PubMed: 16121334]
54. Lin B, White JT, Wu J, et al. Deep depletion of abundant serum proteins reveals low-abundant proteins as potential biomarkers for human ovarian cancer. Proteomics Clin Appl. 2009; 3(7):853–861. [PubMed: 20559449]
55. Qian WJ, Kaleta DT, Petritis BO, et al. Enhanced detection of low abundance human plasma proteins using a tandem IgY12-SuperMix immunoaffinity separation strategy. Mol Cell Proteomics. 2008; 7(10):1963–1973. [PubMed: 18632595]
56▪. Mor G, Visintin I, Lai Y, et al. Serum protein markers for early detection of ovarian cancer. Proc Natl Acad Sci USA. 2005; 102(21):7677–7682. The preclinical basis of the OvaSure™ test is described. [PubMed: 15890779]

57. Amonkar SD, Bertenshaw GP, Chen TH, et al. Development and preliminary evaluation of a multivariate index assay for ovarian cancer. PLoS ONE. 2009; 4(2):E4599. [PubMed: 19240799]

58▪. Edgell T, Martin-Roussety G, Barker G, et al. Phase II biomarker trial of a multimarker diagnostic for ovarian cancer. J Cancer Res Clin Oncol. 2010; 136(7):1079–1088. Phase II biomarker panel analysis. [PubMed: 20082099]

59▪▪. Skates SJ, Horick N, Yu Y, et al. Preoperative sensitivity and specificity for early-stage ovarian cancer when combining cancer antigen CA-125II, CA 15–3, CA 72–4, and macrophage colony-stimulating factor using mixtures of multivariate normal distributions. J Clin Oncol. 2004; 22(20):4059–4066. Multicenter evaluation of candidate biomarker panels. [PubMed: 15381683]

60. Visintin I, Feng Z, Longton G, et al. Diagnostic markers for early detection of ovarian cancer. Clin Cancer Res. 2008; 14(4):1065–1072. [PubMed: 18258665]

61▪. Zhang Z, Bast RC Jr, Yu Y, et al. Three biomarkers identified from serum proteomic analysis for the detection of early stage ovarian cancer. Cancer Res. 2004; 64(16):5882–5890. Serum biomarker panel identified through proteomic methods. [PubMed: 15313933]

62▪. Kurman RJ, Shih IeM. Pathogenesis of ovarian cancer: lessons from morphology and molecular biology and their clinical implications. Int J Gynecol Pathol. 2008; 27(2):151–160. Comprehensive presentation of the two pathological types of ovarian cancer. [PubMed: 18317228]

63. Shin H, Sheu B, Joseph M, Markey MK. Guilt-by-association feature selection: identifying biomarkers from proteomic profiles. J Biomed Inform. 2008; 41(1):124–136. [PubMed: 17544868]

64. Cramer DW, Bast RC Jr, Berg CD, et al. Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prev Res (Phila). 2011; 4(3):365–374. [PubMed: 21372036]

65. Diamandis EP. Cancer biomarkers: can we turn recent failures into success? J Natl Cancer Inst. 2010; 102(19):1462–1467. [PubMed: 20705936]

66. Levanon K, Ng V, Piao HY, et al. Primary *ex vivo* cultures of human fallopian tube epithelium as a model for serous ovarian carcinogenesis. Oncogene. 2010; 29(8):1103–1113. [PubMed: 19935705]

67. Faca VM, Ventura AP, Fitzgibbon MP, et al. Proteomic analysis of ovarian cancer cells reveals dynamic processes of protein secretion and shedding of extracellular domains. PLoS ONE. 2008; 3(6):E2425. [PubMed: 18560578]

68. Kozak KR, Su F, Whitelegge JP, Faull K, Reddy S, Farias-Eisner R. Characterization of serum biomarkers for detection of early stage ovarian cancer. Proteomics. 2005; 5(17):4589–4596. [PubMed: 16237736]

69. Gaytan F, Morales C, Barreiro ML, et al. Expression of growth hormone secretagogue receptor type 1a, the functional ghrelin receptor, in human ovarian surface epithelium, mullerian duct derivatives, and ovarian tumors. J Clin Endocrinol Metab. 2005; 90(3):1798–1804. [PubMed: 15585554]

70. Heppner KJ, Matrisian LM, Jensen RA, Rodgers WH. Expression of most matrix metalloproteinase family members in breast cancer represents a tumor-induced host response. Am J Pathol. 1996; 149(1):273–282. [PubMed: 8686751]

71. Shima I, Sasaguri Y, Kusukawa J, et al. Production of matrix metalloproteinase 9 (92-kDa gelatinase) by human oesophageal squamous cell carcinoma in response to epidermal growth factor. Br J Cancer. 1993; 67(4):721–727. [PubMed: 8471429]

72. Levina VV, Nolen B, Su Y, et al. Biological significance of prolactin in gynecologic cancers. Cancer Res. 2009; 69(12):5226–5233. [PubMed: 19491263]

73. Turner GA, Goodarzi MT, Thompson S. Glycosylation of alpha-1-proteinase inhibitor and haptoglobin in ovarian cancer: evidence for two different mechanisms. Glycoconj J. 1995; 12(3): 211–218. [PubMed: 7496134]

74. Partridge EA, Le Roy C, Di Guglielmo GM, et al. Regulation of cytokine receptors by Golgi N-glycan processing and endocytosis. Science. 2004; 306(5693):120–124. [PubMed: 15459394]

75. Saldova R, Royle L, Radcliffe CM, et al. Ovarian cancer is associated with changes in glycosylation in both acute-phase proteins and IgG. Glycobiology. 2007; 17(12):1344–1356. [PubMed: 17884841]
76. Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ, Van Dijk E, Meloen RH. Monitoring health by values of acute phase proteins. Acta Histochem. 2006; 108(3):229–232. [PubMed: 16714050]
77. Ceciliani F, Giordano A, Spagnolo V. The systemic reaction during inflammation: the acute-phase proteins. Protein Pept Lett. 2002; 9(3):211–223. [PubMed: 12144517]
78. Pritzker KP. Cancer biomarkers: easier said than done. Clin Chem. 2002; 48(8):1147–1150. [PubMed: 12142366]
79. Diamandis EP, Van Der Merwe DE. Plasma protein profiling by mass spectrometry for cancer diagnosis: opportunities and limitations. Clin Cancer Res. 2005; 11(3):963–965. [PubMed: 15709159]
80. Diamandis EP. Proteomic patterns in serum and identification of ovarian cancer. Lancet. 2002; 360(9327):170. author reply 170–171. [PubMed: 12126845]
81. Diamandis EP. Analysis of serum proteomic patterns for early cancer diagnosis: drawing attention to potential problems. J Natl Cancer Inst. 2004; 96(5):353–356. [PubMed: 14996856]
82. Slattery ML, Fitzpatrick FA. Convergence of hormones, inflammation, and energy-related factors: a novel pathway of cancer etiology. Cancer Prev Res (Phila). 2009; 2(11):922–930. [PubMed: 19892662]
83. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J. 1996; 313(Pt 1):17–29. [PubMed: 8546679]
84. Bartsch H, Nair J. Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer: role of lipid peroxidation, DNA damage, and repair. Langenbecks Arch Surg. 2006; 391(5):499–510. [PubMed: 16909291]
85. O'byrne KJ, Dalgleish AG. Chronic immune activation and inflammation as the cause of malignancy. Br J Cancer. 2001; 85(4):473–483. [PubMed: 11506482]
86. Naldini A, Pucci A, Bernini C, Carraro F. Regulation of angiogenesis by Th1- and Th2-type cytokines. Curr Pharm Des. 2003; 9(7):511–519. [PubMed: 12570799]
87. Aldinucci D, Poletto D, Nanni P, et al. CD40L induces proliferation, self-renewal, rescue from apoptosis, and production of cytokines by CD40-expressing AML blasts. Exp Hematol. 2002; 30(11):1283–1292. [PubMed: 12423681]
88. Ono M. Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. Cancer Sci. 2008; 99(8):1501–1506. [PubMed: 18754859]
89. Culig Z. Cytokine disbalance in common human cancers. Biochim Biophys Acta. 2011; 1813(2): 308–314. [PubMed: 21167870]
90. Kundu JK, Surh YJ. Inflammation: gearing the journey to cancer. Mutat Res. 2008; 659(1–2):15–30. [PubMed: 18485806]
91. Lee JM, Yanagawa J, Peebles KA, Sharma S, Mao JT, Dubinett SM. Inflammation in lung carcinogenesis: new targets for lung cancer chemoprevention and treatment. Crit Rev Oncol Hematol. 2008; 66(3):208–217. [PubMed: 18304833]
92. Sharma RA, Dalgleish AG, Steward WP, O'Byrne KJ. Angiogenesis and the immune response as targets for the prevention and treatment of colorectal cancer (review). Oncol Rep. 2003; 10(5): 1625–1631. [PubMed: 12883750]
93. Kikuchi T, Abe T, Yaekashiwa M, et al. Secretory leukoprotease inhibitor augments hepatocyte growth factor production in human lung fibroblasts. Am J Respir Cell Mol Biol. 2000; 23(3):364–370. [PubMed: 10970828]
94. Dabbagh K, Laurent GJ, Shock A, Leoni P, Papakrivopoulou J, Chambers RC. Alpha-1-antitrypsin stimulates fibroblast proliferation and procollagen production and activates classical MAP kinase signalling pathways. J Cell Physiol. 2001; 186(1):73–81. [PubMed: 11147816]
95. Nasulewicz A, Mazur A, Opolski A. Role of copper in tumour angiogenesis – clinical implications. J Trace Elem Med Biol. 2004; 18(1):1–8. [PubMed: 15487757]

96. Zhu CS, Pinsky PF, Cramer DW, et al. A framework for evaluating biomarkers for early detection: validation of biomarker panels for ovarian cancer. Cancer Prev Res (Phila). 2011; 4(3):375–383. [PubMed: 21372037]
97. Lafky JM, Wilken JA, Baron AT, Maihle NJ. Clinical implications of the ErbB/epidermal growth factor (EGF) receptor family and its ligands in ovarian cancer. Biochim Biophys Acta. 2008; 1785(2):232–265. [PubMed: 18291115]
98. Su F, Kozak KR, Imaizumi S, et al. Apolipoprotein A-I (apoA-I) and apoA-I mimetic peptides inhibit tumor development in a mouse model of ovarian cancer. Proc Natl Acad Sci USA. 2010; 107(46):19997–20002. [PubMed: 21041624]
99. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Royal Stat Soc B (Methodol). 1995; 57(1):289–300.
100. Castracane VD, Gimpel T, Goldzieher JW. Diagnosis of menopause: ratios of FSH/LH? Fertil Steril. 1996; 66(5):866. [PubMed: 8893709]
101. Czygan PJ. Plasma levels of FSH and LH during the menstrual cycle, the menopause and in the course of gestagen treatment. Acta Endocrinol Suppl (Copenh). 1971; 152:2. [PubMed: 4101326]
102. Andersen TT, Curatolo LM, Reichert LE Jr. Follitropin binding to receptors in testis: studies on the reversibility and thermodynamics of the reaction. Mol Cell Endocrinol. 1983; 33(1):37–52. [PubMed: 6315509]
103. Moore LE, Fung ET, Mcguire M, et al. Evaluation of apolipoprotein A1 and posttranslationally modified forms of transthyretin as biomarkers for ovarian cancer detection in an independent study population. Cancer Epidemiol Biomarkers Prev. 2006; 15(9):1641–1646. [PubMed: 16985025]
104. Nosov V, Su F, Amneus M, et al. Validation of serum biomarkers for detection of early-stage ovarian cancer. Am J Obstet Gynecol. 2009; 200(6):639, e631–e635. [PubMed: 19285648]
105. Levina V, Nolen BM, Marrangoni AM, et al. Role of eotaxin-1 signaling in ovarian cancer. Clin Cancer Res. 2009; 15(8):2647–2656. [PubMed: 19351767]
106. Abbott KL, Lim JM, Wells L, Benigno BB, Mcdonald JF, Pierce M. Identification of candidate biomarkers with cancer-specific glycosylation in the tissue and serum of endometrioid ovarian cancer patients by glycoproteomic analysis. Proteomics. 2010; 10(3):470–481. [PubMed: 19953551]
107. Mirshahi M, Soria J, Soria C, Bertrand O, Basdevant A. Glycosylation of human fibrinogen and fibrin *in vitro*. Its consequences on the properties of fibrin(ogen). Thromb Res. 1987; 48(3):279–289. [PubMed: 3124289]
108▪. Kurman RJ, Shih IeM. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer – shifting the paradigm. Hum Pathol. 2011; 42(7):918–931. Current review of the molecular and pathological heterogeneity of ovarian cancer. [PubMed: 21683865]
109. De Bruijn HW, Ten Hoor KA, Boonstra H, Marrink J, Krans M, Aalders JG. Cancer-associated antigen CA 195 in patients with mucinous ovarian tumours: a comparative analysis with CEA, TATI and CA 125 in serum specimens and cyst fluids. Tumour Biol. 1993; 14(2):105–115. [PubMed: 8392219]
110. Brun JL, Cortez A, Commo F, Uzan S, Rouzier R, Darai E. Serous and mucinous ovarian tumors express different profiles of MMP-2, -7, -9, MT1-MMP, and TIMP-1 and -2. Int J Oncol. 2008; 33(6):1239–1246. [PubMed: 19020757]
111. Murthi P, Barker G, Nowell CJ, et al. Plasminogen fragmentation and increased production of extracellular matrix-degrading proteinases are associated with serous epithelial ovarian cancer progression. Gynecol Oncol. 2004; 92(1):80–88. [PubMed: 14751142]
112. Adley BP, Gleason KJ, Yang XJ, Stack MS. Expression of membrane type 1 matrix metalloproteinase (MMP-14) in epithelial ovarian cancer: high level expression in clear cell carcinoma. Gynecol Oncol. 2009; 112(2):319–324. [PubMed: 18976802]
113. Shigemasa K, Tanimoto H, Sakata K, et al. Induction of matrix metalloprotease-7 is common in mucinous ovarian tumors including early stage disease. Med Oncol. 2000; 17(1):52–58. [PubMed: 10713661]
114. Lu L, Katsaros D, Wiley A, et al. Promoter-specific transcription of insulin-like growth factor-II in epithelial ovarian cancer. Gynecol Oncol. 2006; 103(3):990–995. [PubMed: 16859738]

115. Lu L, Katsaros D, Wiley A, et al. The relationship of insulin-like growth factor-II, insulin-like growth factor binding protein-3, and estrogen receptor-alpha expression to disease progression in epithelial ovarian cancer. Clin Cancer Res. 2006; 12(4):1208–1214. [PubMed: 16489075]

116. Mabuchi S, Kawase C, Altomare DA, et al. Vascular endothelial growth factor is a promising therapeutic target for the treatment of clear cell carcinoma of the ovary. Mol Cancer Ther. 2010; 9(8):2411–2422. [PubMed: 20663925]

117. Kawamura K, Komohara Y, Takaishi K, Katabuchi H, Takeya M. Detection of M2 macrophages and colony-stimulating factor 1 expression in serous and mucinous ovarian epithelial tumors. Pathol Int. 2009; 59(5):300–305. [PubMed: 19432671]

118. Kobel M, Kalloger SE, Boyd N, et al. Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS Med. 2008; 5(12):e232. [PubMed: 19053170]

119. Su F, Lang J, Kumar A, et al. Validation of candidate serum ovarian cancer biomarkers for early detection. Biomark Insights. 2007; 2:369–375. [PubMed: 19662218]

120. Kurman RJ, Visvanathan K, Roden R, Wu TC, Shih IeM. Early detection and treatment of ovarian cancer: shifting from early stage to minimal volume of disease based on a new model of carcinogenesis. Am J Obstet Gynecol. 2008; 198(4):351–356. [PubMed: 18395030]

121▪. Lu D, Kuhn E, Bristow RE, et al. Comparison of candidate serologic markers for type I and type II ovarian cancer. Gynecol Oncol. 2011; 122(3):560–566. Comparative analysis of serum biomarkers associated with types I and II ovarian cancer. [PubMed: 21704359]

122. Greene MH, Feng Z, Gail MH. The importance of test positive predictive value in ovarian cancer screening. Clin Cancer Res. 2008; 14(22):7574. author reply 7577–7579. [PubMed: 18948386]

123. Mcintosh M, Anderson G, Drescher C, et al. Ovarian cancer early detection claims are biased. Clin Cancer Res. 2008; 14(22):7574. author reply 7577–7579. [PubMed: 18948385]

124▪. Havrilesky LJ, Whitehead CM, Rubatt JM, et al. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. Gynecol Oncol. 2008; 110(3): 374–382. Review of transcriptional profiling efforts aimed at ovarian cancer biomarker discovery. [PubMed: 18584856]

125. Palmer C, Duan X, Hawley S, et al. Systematic evaluation of candidate blood markers for detecting ovarian cancer. PLoS ONE. 2008; 3(7):e2633. [PubMed: 18612378]

126. Anderson GL, Mcintosh M, Wu L, et al. Assessing lead time of selected ovarian cancer biomarkers: a nested case–control study. J Natl Cancer Inst. 2010; 102(1):26–38. [PubMed: 20042715]

127▪▪. Andersen MR, Goff BA, Lowe KA, et al. Use of a Symptom Index, CA125, and HE4 to predict ovarian cancer. Gynecol Oncol. 2010; 116(3):378–383. Prospective evaluation of biomarker panel combined with Symptom Index in prediagnostic samples. [PubMed: 19945742]

128. Gohagan JK, Prorok PC, Hayes RB, Kramer BS. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial of the National Cancer Institute: history, organization, and status. Control Clin Trials. 2000; 21(Suppl 6):251S–272S. [PubMed: 11189683]

129. Folkins AK, Jarboe EA, Roh MH, Crum CP. Precursors to pelvic serous carcinoma and their clinical implications. Gynecol Oncol. 2009; 113(3):391–396. [PubMed: 19237187]

130. Pinho S, Marcos NT, Ferreira B, et al. Biological significance of cancer-associated sialyl-Tn antigen: modulation of malignant phenotype in gastric carcinoma cells. Cancer Lett. 2007; 249(2):157–170. [PubMed: 16965854]

131. Bast RC Jr. Status of tumor markers in ovarian cancer screening. J Clin Oncol. 2003; 21(Suppl 10):200s–205s. [PubMed: 12743135]

132. Moore RG, Maclaughlan S. Current clinical use of biomarkers for epithelial ovarian cancer. Curr Opin Oncol. 2010; 22(5):492–497. [PubMed: 20613519]

133. Pitteri SJ, Jebailey L, Faca VM, et al. Integrated proteomic analysis of human cancer cells and plasma from tumor bearing mice for ovarian cancer biomarker discovery. PLoS ONE. 2009; 4(11):E7916. [PubMed: 19936259]

134. Gadducci A, Ferdeghini M, Castellani C, et al. Serum levels of tumor necrosis factor (TNF), soluble receptors for TNF (55- and 75-kDa sTNFr), and soluble CD14 (sCD14) in epithelial ovarian cancer. Gynecol Oncol. 1995; 58(2):184–188. [PubMed: 7542621]

135. Naldini A, Morena E, Belotti D, Carraro F, Allavena P, Giavazzi R. Identification of thrombin-like activity in ovarian cancer associated ascites and modulation of multiple cytokine networks. Thromb Haemost. 2011; 106(4):705–711. [PubMed: 21833453]

136. Morin PJ. Claudin proteins in ovarian cancer. Dis Markers. 2007; 23(5–6):453–457. [PubMed: 18057528]

137. Pyle-Chenault RA, Stolk JA, Molesh DA, et al. VSGP/F-spondin: a new ovarian cancer marker. Tumour Biol. 2005; 26(5):245–257. [PubMed: 16103746]

138. Simon I, Liu Y, Krall KL, et al. Evaluation of the novel serum markers B7-H4, Spondin 2, and DcR3 for diagnosis and early detection of ovarian cancer. Gynecol Oncol. 2007; 106(1):112–118. [PubMed: 17490732]

139. Dupont J, Tanwar MK, Thaler HT, et al. Early detection and prognosis of ovarian cancer using serum YKL-40. J Clin Oncol. 2004; 22(16):3330–3339. [PubMed: 15310777]

140. Hogdall E, Fung ET, Christensen IJ, et al. Proteomic biomarkers for overall and progression-free survival in ovarian cancer patients. Proteomics Clin Appl. 2010; 4(12):940–952. [PubMed: 21137034]

141. Cho H, Kim JH. Lipocalin2 expressions correlate significantly with tumor differentiation in epithelial ovarian cancer. J Histochem Cytochem. 2009; 57(5):513–521. [PubMed: 19188485]

142. Tsukishiro S, Suzumori N, Nishikawa H, Arakawa A, Suzumori K. Use of serum secretory leukocyte protease inhibitor levels in patients to improve specificity of ovarian cancer diagnosis. Gynecol Oncol. 2005; 96(2):516–519. [PubMed: 15661245]

143. Strieter RM, Burdick MD, Mestas J, Gomperts B, Keane MP, Belperio JA. Cancer CXC chemokine networks and tumour angiogenesis. Eur J Cancer. 2006; 42(6):768–778. [PubMed: 16510280]

144. Gorelik E, Landsittel DP, Marrangoni AM, et al. Multiplexed immunobead-based cytokine profiling for early detection of ovarian cancer. Cancer Epidemiol Biomarkers Prev. 2005; 14(4): 981–987. [PubMed: 15824174]

Website

201. National Cancer Institute. SEER Cancer Statistics Review, 1975–2007. 2010. http://seer.cancer.gov/csr/1975_2007/

Executive summary

Background

- Ovarian cancer is the most lethal gynecological malignancy.
- Ovarian cancer is characterized by nonspecific symptomatic presentation, complicating early detection.
- A shift in diagnosis from late- to early-stage disease could lead to a significant increase in survival.

Screening for early disease: requirements & reality

- Current methods utilized in the detection of ovarian cancer are inadequate for use as population screening tools.
- The CA-125 blood test is widely utilized but is characterized by several limitations.
- Two large prospective clinical trials investigating ovarian cancer screening (PLCO and UKCTOCS) have reported mixed findings.
- An expanded analysis of subtype-specific biomarker alterations, including specific precursors of those subtypes, should lead to improved biomarker discovery and development.

Ovarian cancer screening biomarkers: the forest

- The Early Detection Research Network has outlined five phases required for successful biomarker development.
- A large number of candidate biomarkers for ovarian cancer have been identified in several bodily fluids, primarily serum, through the use of multiple technological platforms.
- Multiplexed platforms permit the evaluation of large arrays of potential biomarkers at minimal time and sample volume requirements.

Individual biomarkers of ovarian cancer: the trees

- Our review identified 160 proteins altered in the sera of ovarian cancer patients and healthy controls.
- The list of candidate biomarkers can be broken down on the basis of biological properties into the following functional groups presented in order of predominance: mediators of inflammation; acute-phase reactants; adhesion molecules and proteases; hormones; and growth and angiogenesis factors.
- The multiple functional groups represented by the array of biomarkers may be indicative of the multiple tumorigenic pathways involved in the development of ovarian cancer.

Feature selection

- In the selection of candidate biomarkers for inclusion in diagnostic multimarker panels, proteins should be first prioritized based on the following biological characteristics: measurable alterations in noninvasive samples; informative for all histological and pathological subtypes; and temporal reliability.
- Specific considerations for biomarker selection include biomarker specificity for the various histological subtypes of ovarian cancer; biomarker specificity for

type I and type II ovarian cancers; nonclassical biomarkers; and proteins observed to be decreased in ovarian cancer patients and those produced by extratumoral sources.

- Bioinformatic feature selection algorithms can be divided into three categories: filters, wrappers and embedded.

Approaches & controversies in biologically based feature selection

- Several trends in ovarian cancer biomarker development challenge the premise that biomarkers must arise directly from tumor cells and exhibit elevated serum levels in patients versus controls: stroma-derived biomarkers; acute-phase reactants; and downregulated proteins.

Biomarkers of different subtypes of ovarian cancer

- Specific biomarkers associated with the various histological subtypes of ovarian epithelial cancer may provide complementation and improved performance when assembled into diagnostic panels.
- Biomarkers associated with pathologically defined type II ovarian carcinomas are especially sought after, given the aggressive nature of these tumors.

Selection of multimarker panels based on postdiagnostic samples

- A number of groups have reported promising results based on samples collected at or after the time of diagnosis.
- Methods employed included immunoassay microarray, transcriptional profiling, multiplexed bead-based immunoassay, SELDI-TOF mass spectroscopy and a variety of bioinformatic analysis techniques.

Multimarker panels in prediagnostic samples

- Several biomarkers including CA-125, HE4 and mesothelin have been evaluated in prediagnostic ovarian cancer sample with some indication of efficacy within 1 year of diagnosis.
- A large multisite evaluation of prediagnostic biomarkers in conjunction with the PLCO Screening Trial identified several candidate biomarkers with measurable alterations between ovarian cancer patients and controls.
- No biomarker combination was identified that could outperform CA-125 alone for discrimination between the two groups.

Conclusions

- Preclinical findings suggest that diagnostic panel performance is approaching the level required for clinical advancement; however, large-scale prospective evaluation of selected panels is currently lacking.
- The expanding view of ovarian cancer heterogeneity will likely lead to further refinement of the goals of early detection and the methods employed to achieve those goals.

Table 1

Ovarian cancer-associated proteins.

Biological function	Biomarkers	Role in cancer
Cytokines/chemokines	IL-1, -6, -7, -8, -10, -11, -12, -16, -18, -21, -23, -28A, -33, LIF, TNFR1–2, HVEM (TNFRSF14), IL1R-a, IL1R-b, IL-2R, M-CSF, MIP-1a, TNF-α, CD40, RANTES, CD40L, MIF, IFN-β, MCP-4 (CCL13), MIG (CXCL9), MIP-1δ (CCL15), MIP3a (CCL20), MIP-4 (CCL18), MPIF-1, SDF-1a + b (CXCL12), CD137/4–1BB, lymphotactin (XCL1), eotaxin-1 (CCL11), eotaxin-2 (CCL24), 6Ckine/CCL21), BLC (CXCL13), CTACK (CCL27), BCA-1 (CXCL13), HCC4 (CCL16), CTAP-3 (CXCL7)	Tumor growth, angiogenesis, immune functions, inflammation, invasion
Growth/angiogenic factors	IGF1, VEGF, VEGFR3, EGFR, ErbB2, CTGF, PDGF AA, BB, PDGFRb, bFGF, TGFbRIII, β-cellulin, IGFBP1–4, 6, BDNF, PEDF, angiopoietin-2, renin, lysophosphatidic acid, β2-microglobulin, sialyl TN, ACE	Tumor growth, angiogenesis
Adhesion molecules/proteases/related molecules	CA-125, CA 19–9, CEA, CA 15–3, CA-50, CA 72–4, OVX1, mesothelin, sialyl TN, MMP-2, -3, -7, -9, VAP-1, TIMP1–2, tenascin C, VCAM-1, osteopontin, KIM-1, NCAM, tetranectin, nidogen-2, cathepsin L, prostasin, matriptase, kallikreins 2, 6, 10, cystatin C, claudin, spondin2, SLPI	Invasion/metastases
Hormones/related molecules	bHCG, urinary gonadotropin peptide, inhibin, leptin, adiponectin, GH, TSH, ACTH, PRL, FSH, LH, cortisol, TTR, osteocalcin, insulin, ghrelin, GIP, GLP-1, amylin, glucagon, peptide YY, follistatin, hepcidin	Tumor growth, transformation
Acute-phase reactants/coagulation factors	CRP, Apo A1, CIII, H, transthyretin, SAA, SAP, complement C3,4, complement factor H, albumin, ceruloplasmin, haptoglobin, β-hemoglobin, transferrin, ferritin, fibrinogen, thrombin, von Willebrand factor, myoglobin, immunosuppressive acidic protein, lipid-associated sialic acid, S100A12 (EN-RAGE), fetuin A, clusterin, α1-antitrypsin, a2- macroglobulin, serpin1 (human plasminogen activator inhibitor-1), Cox-1, Hsp27, Hsp60, Hsp80, Hsp90, lectin-type oxidized LDL receptor 1, CD14, lipocalin 2, ITIH4	Inflammation, tumor growth, angiogenesis
Apoptosis factors	sFasL, Cyfra21–1, TPA, perforin, DcR3	Tumor growth, immune functions
Energy homeostasis	AGRP, creatine kinase-MB	Tumor growth
Proteins with undefined or other functions	HE4, human milk fat globule 1–2, NT-Pro-BNP, neuron-specific enolase, CASA, NB/70K, AFP, afamin, collagen, prohibitin, keratin-6, PARC, B7-H4, YK-L40	

Data taken from [40,52,54,56,130–143].

Table 2

Molecular and cellular functions associated with ovarian cancer biomarkers.

Function	p-value	Number of molecules
Cellular movement	6.88×10^{-61}–6.13×10^{-14}	104
Cell-to-cell signaling and interaction	3.85×10^{-51}–4.07×10^{-14}	104
Cellular growth and proliferation	4.20×10^{-51}–3.13×10^{-14}	107
Cell death	3.37×10^{-44}–6.09×10^{-14}	102
DNA replication, recombination and repair	5.19×10^{-32}–2.77×10^{-17}	47

Table 3

Physiological networks associated with ovarian cancer biomarkers.

Network	Biomarkers in network	Score	Top functions
1	A2M, adipokine, angiopoietin, APOA1, APOH, SAP, C3, CCL4, CCL5, CCL11, CCL13, CCL15, CCL18, CCL20, CCL21, CCL24, CCL27, CD14, CD40, CD40L, creatine kinase, CRP, CTGF, CXCL9, CXCL13, EGFR, Erbb2, eotaxin, ferritin, fibrin, fibrinogen, GH, hemoglobin, IFN-β, IGF1, IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP6, IL-1, IL-6, IL-8, IL-10, IL-23, IL-33, IL-1R, IL-12, IL-17, IL-1R1, IL-23A, inhibin, LIF, LPA, MIF, MMP-2, MMP-3, MMP-7, MMP-9, PYY, S100A12, SAA, SAA1, tPAI1, Tenascin, TGF-β, TIMP1, TIMP2, TNF, TNFR1 and 2, TTR, VCAM1, XCL1	115	Cellular movement, hematological system development and function, immune cell trafficking
2	BDNF, CEA, EGFR, ErbB2, FSH, ghelin, GIP, hCG, IL-7, IL-16, IL-2R, IL-2R, insulin, integrin, KLK6, NCAM1, PDGF-BB, PDGF-AA, PRL, TNF, TSH, VEGF, VWF, clusterin, β-cellulin, albumin, glucagon, casein, tansferrin, tenascin C, CXCL12, thrombin, FasL, KIM-1, MFG8	46	DNA replication, recombination and repair
3	Afamin, AGRP, APOC3, CCL13, CCL27, CKB, HGF, IL-6, IL-17, KLK2, KLK11, MUC16, PDGF-DD, complement factor H, AHSG, claudin, collagen, cathepsin D, leptin, mesothelin, nidogen 2, α-antitrypsin, HE4	22	Cell cycle, inflammatory response

Table 4

Multimarker panels that discriminate ovarian cancer cases from healthy controls.

Panel	Cases/controls	SN/SP	Analytical platform	Bioinformatics method	Biomarker prioritization	Year	Ref.
CA-125, ApoA1, TTR	200/142†	74/97	MS	SVM	No	2004	[61]
CA-125, CA 72–4, MCSF	123/224†	70/98	ELISA	CT, MDA, LR	Yes	2004	[59]
CA-125, IL-6, IL-8, VEGF, EGF	44/45	85/95	BBIA	CT	No	2005	[144]
CA-125, TTR, ApoA1	20/82	89/92	MS	LR	No	2007	[119]
CA-125, leptin, PRL, OPN, IGF-II, MIF	156/362†	95.3/99.4	BBIA	LR	No	2008	[60]
CA-125, HE4, glycodelin, cPlau-R, MUC1, PAI1	200/396	80–89/87	BBIA	LR	Yes	2008	[124]
CA-125, HE4, CEA, VCAM-1	456/2000†	86–93/98	BBIA	MMC	No	2010	[54,12]
CA-125, CA 19–9, EGFR, CRP, myoglobin, Apo A1, CIII, MIP1a, IL-6, IL-18, tenascin C	176/187†	91.3/88.5	BBIA	Knowledge Discovery Engine-VS	No	2008, 2009	[52,57]
CA-125, CRP, SAA, IL-6, IL-8	150/212†	94.1/91.3	BBIA	LR	Yes	2010	[58]
CA-125, HE4, SI	74/137	84/98.5	ELISA	N/A	Yes	2010	[127]

† Includes independent validation set.

BBIA: Bead-based immunoassay; CT: Classification trees; LR: Logistic regression; MDA: Mixture discriminant analysis; MMC: Metropolis-Monte Carlo algorithm; MS: Mass spectroscopy; N/A: Not applicable; SI: Symptom index; SN: Sensitivity; SP: Specificity; SVM: Support vector machine.

Table 5

Trends in serum biomarker alteration in three large studies.

Biological function	Trend in OC	Biomarkers
Cytokines/chemokines	Elevated	IL-10†, IL-6†, TNF-α†, TNFR2†, 6Ckine, BCA-1, BLC/CXCL, CCL20/MIP3α, CD40, CXCL9/MIG, IL-1Rα, IL-11, IL-16, IL-1β, IL-21, IL-28A, IL-2R, IL-33, IL-7, IL-8, LIF, MIF, MIP-1δ, RANTES, sCD137/4, TNFR1
	Decreased	Eotaxin-1, eotaxin-2, IL-18, macrophage-derived chemokine, XCL1/ lymphotactin
Growth/angiogenic factors	Elevated	IGFBP1†, angiopoietin-2, β2-microglobulin, β-cellulin, CTGF, bFGF, heparin-binding EGF-like growth factor, IGFBP2, IGFBP4, PDGF, VEGF
	Decreased	EGFR‡, BDNF†, ErbB2†, IGFBP3, IGFBP6, IGF-I, PEDF, stem cell factor
Adhesion molecules/proteases	Elevated	CA-125†, CA 19–9†, OPN†, TIMP-1†, CA 15–3, CA 72–4, cathepsin D, MMP-9, tenascin C
	Decreased	VCAM-1†, MMP-2, MMP-3, NCAM
Hormones	Elevated	ACTH, GH, GLP-1, prolactin, TSH
	Decreased	Adiponectin, estrogen receptor-α, FSH, ghrelin, GIP, insulin, leptin, LH, osteocalcin, thrombopoietin
Acute-phase reactants/coagulation factors	Elevated	α1-antitrypsin†, CRP†, EN-RAGE, ferritin, fibrinogen, haptoglobin, HSA, PAI-1, protein S100-A12 (EN-RAGE), SAP, von Willebrand factor
	Decreased	α2-macroglobulin†, ApoA1†, ApoCIII, ApoH, clusterin, complement C3, C4, factor VII, fetuin A, prealbumin†
Apoptosis factors	Elevated	Cyfra 21–1
	Decreased	Fas, perforin
Energy homeostasis	Elevated	AGRP
	Decreased	Creatine kinase-MB
Undefined or other functions	Elevated	Anti-TFF-3, FABP, HE4, NSE, NT-Pro-BNP
	Decreased	None

†Trend agreement in two studies.

‡Trend agreement in three studies.

EGFR: EGF receptor.

Data taken from [51,52] [Nolen LBM, Lokshin AE, Unpublished Data].