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Blood and urine markers for ovarian cancer: A comprehensive review[1]

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1. Introduction

The obvious appeal of a strategy for the early detection of ovarian cancer is based upon the tendency for the disease to present at advanced stages associated with poor survival. If the diagnosis could be largely shifted to stage I associated with survival close to 90%, then the overall mortality for this disease could be dramatically altered without any advances in therapy. Whether a screening strategy can be successfully implemented for ovarian cancer depends upon the natural history of the disease, especially the length of time ovarian cancer remains in a pre-clinical phase prior to stage I, and the ability to develop a convenient and suitably-performing screening test acceptable to the target population. Pre-clinical disease refers to a stage in a disease process before which the disease has produced symptoms, which lead an individual to seek diagnosis or treatment. In the case of cancers like cervix or colon, pre-clinical disease can be equated with well-characterized precursor lesions known to precede the invasive cancer. But, for ovarian cancer, precursor lesions are largely unknown as is the length of time that the disease remains in a pre-clinical phase.

Despite this lack of understanding, the public health importance of ovarian cancer demands active investigation of approaches to screening. Although these approaches might include pelvic examination, sonography, or even cytologic approaches, this review will focus on blood or urine markers for ovarian cancers as methods most likely to be adaptable to screening both general and high-risk populations. Table 1 summarizes ovarian cancer markers for which sensitivity and specificity estimates were available or where mean levels of markers for cases and controls were available. Sensitivity, the proportion of all those with disease who are screen positive, and specificity, the proportion of all those without disease who are screen negative, are chosen as the key performance characteristics. Unlike related measures such as the predictive values of a positive or negative test, which depend upon the prevalence of the disease in the population, sensitivity and specificity are more stable test parameters. We have also attempted to organize markers by structural or functional characteristics in order that some generalizations about broader categories of markers may be possible, acknowledging that there may be disagreement about how best to categorize a particular marker.

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2. Epithelial sialomucins

The epithelial sialomucins are currently the most important group of markers for ovarian cancer. These are heavily glycosylated proteins with relatively high molecular weights and include the only approved marker for monitoring ovarian cancer, CA-125, also known as MUC16. Although these proteins are transmembrane in location, they can be found in the serum. Most of the markers included in this category have been identified through approaches in which human cancer cells are used as an antigenic stimulus in animals to raise antibodies, which can then efficiently detect the antigen in human serum. A review of Table 1 suggests that CA 125 remains the best-documented and best-performing single marker among the epithelial sialomucins currently described [2,6,47,48,54,63,91,94,98, 101,102,108,112,116]. Sensitivity estimates vary from as low as 27% in some studies for early-staged disease to better than 90% for late-staged disease. Sensitivity is also greater for serous and endometrioid ovarian tumors compared to mucinous or clear cell tumors.

CA 15-3 (MUC1) is a biomarker described for breast (and other cancers) but is also relevant to ovarian cancer. It appears to be expressed in ovarian cancer types similar to CA 125 with promising sensitivity and specificity estimates in limited studies [7,24,48,80,102]. The nomenclature on CA 15-3 is confusing with a number of other terms describing markers similar or identical to CA 15-3 including human milk fat globulin 1 and 2 (HMFG1/2), CASA, OSA, and several others. The remaining sialomucins listed in Table 1 are not serious candidates to replace CA 125 based upon usually poorer and certainly more limited sensitivity and specificity estimates. Rather these are better viewed as markers, which may prove complementary to CA 125. Thus CA 50, CA 54-61, CA 195, and CA 19-9 all appear to have greater sensitivity for mucinous tumors [17,29, 47,88] while STN and TAG-72 have better sensitivity for clear cell tumors [31,54]. Combinations of markers involving the sialomucins are discussed in more detail in Section 12.

In general there are several limitations common to members of this family of markers. First, based on their molecular weight, urine would not be a good medium to use in screening for these markers. Secondly, the high molecular weight and differing glycosylation patterns (epitopes) combine to produce the diagnostic problem that different antibodies can be generated and contribute to a lack of consistency between assays using different antibodies [99]. Thirdly, these markers may be secreted by a variety of epithelial tumors including breast, colon, ovary and pancreas as well as normal adult epithelial tissue including that from the breast, gastrointestinal and genitourinary tracts. Finally, a number of benign conditions can be associated with elevation of these markers. In particular, CA125 can be elevated in early pregnancy and with endometriosis, fibroids, or infections of the genital tract [99]. These would, of course, affect the specificity of the test and the possibility of false positive results. One strategy, besides combining markers, to improve the specificity associated with use of these markers would be to use serial testing. Elevated but declining marker levels would indicate a transient condition associated with marker production. Elevated but stable levels might indicate chronic but benign conditions associated with marker production, while elevated and increasing levels are more likely indicative of cancer [87,112]. Another approach to improve specificity would be to combine the marker with an imaging test such as pelvic ultrasound– a strategy found in a randomized trial in England to improve the median survival of screen detected cases [113].

3. Proteases, inhibitors, and cleavage products

Based on the number described, the proteases and related peptides may be the next most valuable group of markers for ovarian cancer. Within this group, the kallikreins, a family of serine proteases, have captured attention because of the importance of prostate-specific antigen (PSA) for detecting prostate cancer in men. PSA was originally discovered through techniques similar to those employed for the sialomucins in which crude or partially purified extracts of normal and cancerous prostate tissues were used to immunize rabbits [100]. Later, PSA was identified to be a member of the human kallikrein family prompting interest in other members of this group. The importance of this family of proteins to ovarian cancer has been subsequently demonstrated through techniques used to identify genes, which are over expressed in ovarian cancer cells. Indeed, the entire family of kallikreins map to a region on chromosome 19q shown to be amplified in ovarian cancers [19,20,85,110].

Currently the best performing of these markers include kallikrein 6 (protease M) and kallikrein 10 with sensitivities reported between 47 and 75% and specificities between 90 and 100% [21,56]. Sensitivity was improved when kallikrein 10 was combined with CA

Table 1
Potential ovarian cancer tumor markers with previously reported sensitivity and specificities

Category/Marker	References	Sensitivity	Specificity	Comments
Epithelial sialomucins				
CA 125 (MUC 16)	Adonkis 1996[a], Bast 1983[a], Inoue 1992[a,b], Jacobs 1992[a,b], Kudoh 1999[a], Molina 1992[a], Tholander 1990[a,b], Tamakoshi 1996, van Haaften-Day 2001 Woolas 1995[a], Woolas 1993[a], Yin 2001, Zurawski 1990[a]	24–97%	71–100%; 62% in subjects with other malignancies; 71–86% in benign tumors; 96% in healthy controls	Sensitivity by stage: Stage I = 27–66%, Stage II = 65–100%; Stage III = 87–90% Stage IV = 94% Sensitivity by histology: Serous: 68–94.1% Mucinous: 52–68% Endometrioid: 92%, Clear cell: 61%;
CA 15-3 (MUC-1)	Bast 1991[a], Feng 2002, Jacobs 1992[a], Scambia 1988[a], Woolas 1995[a]	57–93%	80% in women with benign ovarian tumors; 100% in women with benign gynecological conditions; 87% in women with pelvic mass; 98% in women with benign disease and elevated CA 125	Sensitivity by stage: Stage I = 27%, Stage II = 79–87%, Stage III = 85–93% MUC-1 expression less common in mucinous tumors, more common in serous, endometrioid; In combination with TAG 72.3 or CA 125, specificity ranges from 94–100%;
HMFG1/G2	Bast 1991[a], Dhokia 1986[b], Fisken 1993[b],	50–65%	83–97%; 12–26% in women with benign disease and elevated CA 125	Sensitivity by stage: Stage I: 45%, Stage II 54%, Stage III: 61%, Stage IV: 75%; Sensitivity by histology: Serous: 51–68% Mucinous: 25% Clear cell: 40–60% Endometrioid: 58–75%; In combination with CA125, sensitivity = 95%, specificity = 93%
Cancer-associated serum antigen (CASA)	McGuckin 1990[a,b], Hogdall 2000[a]	30–76%	98% in benign tumors and normal controls	Sensitivity by stage: Stage I/II: 23%, Stage III/IV: 39%
CA 50	Gadducci 1991[a]	35%	67%	Sensitivity in non-mucinous = 26%, Sensitivity in mucinous = 88%
CA 54-61	Suzuki 1990, Woolas 1995[a]	52–60%	88–95%	Sensitivity in mucinous = 78%,
CA 195 (carbohydrate antigen 195)	de Bruijn 1993[a], Gadducci 1991[a]	35–72%	72–86%	Sensitivity in mucinous = 72%, Sensitivity in non-mucinous = 35%
CA 19-9	Engelen 2000[a], Inoue 1992[a,b], Kudoh 1999[a], Molina 1992[a], Gadducci 1991[a], Tamakoshi 1996, Woolas 1995[a]	24–75%	59% in benign tumors; 52% in subjects with other malignancies; 88% in women with pelvic mass	Sensitivity by stage: Stage I = 33–62%, Stage II = 10–60%, Stage III = 35%, Stage IV = 41%; Sensitivity by histology: Serous: 40–52% Mucinous: 45–80% Endometrioid: 50–75% Clear cell: 60.9%
MAM-6	Hilkens 1986	78%	97% in subjects with benign tumors (all kinds)	Mean level in ovarian cancer patients: 43.2 ± 64 units/ml
NB/70K	Bast 1991[a], Petru 1990[a,b], Knauf 1988[b],	57–76%	74%; 38% in women with benign disease and elevated CA 125	45–50% sensitivity in early stage disease
Ovarian serum antigen (OSA)	McGuckin 1990[a]	82%	95%	

Table 1, continued

Category/Marker	References	Sensitivity	Specificity	Comments
OVX1	Hogdall 2000[a], van Haaften-Day 2001, Woolas 1993[a], Woolas 1995[a], Xu 1993	9–70%	83–94%; 86–93% in benign, 91–95% healthy controls	Sensitivity by stage: Stage I: 22%, Stage II: 25%, Stage III: 15%, Stage IV: 18%; Sensitivity by histology: Serous: 70%, Mucinous: 75%, Endometrioid: 67% Incombination with CA 125 and MCSF, sensitivity = 85%, specificity = 83%
STN (sialyl TN)	Inoue 1990[b], Inoue 1992[a,b], Kudoh 1999[a],	44–50%	92% in benign tumors; 94% in benign ovarian cysts; 99% in normal volunteers	Sensitivity by stage: Stage I: 31–44%, Stage II: 29–50% Stage III: 69% Sensitivity by histology: Serous: 61.9% Mucinous: 60.0%, Endometrioid: 60.0% Clear cell: 75.0%
TAG-72 (tumor associated glycoprotein-72) (called CA72-4)	Bast 1991[a], Fiella 1999, Guadagni 1994[a], Jacobs 1992[a,b], Negishi 1993, Nishida 1995[a], Woolas 1995[a], Zeimet 1995	10–63%	73–99%; 94% in women with benign disease and elevated CA125; 97% in benign ovarian conditions, 100% in other benign gynecologic conditions	Sensitivity by stage: Stage I-II: 10% Stage III-IV: 56% Sensitivity by histology: Serous: 59%, Mucinous: 25%, Endometrioid: 63%, May be more useful in clear cell tumors; In combination with CA 125, sensitivity = 86%, spec. = 83%; In combination with TAG 72.3 or CA 15-3, specificity ranges from 94–100%;
Proteases and their inhibitors				
Kallikrein 6 (Protease M)	Diamandis 2000[b], Diamandis 2003[a,b]	47–66%	100% in healthy men and women; 90–95% in healthy and benign controls	Sensitivity by stage: Stage I: 16% Stage II: 27% Stage III: 75% Stage IV: 63% Sensitivity by histology: Serous: 68% Endometrioid 33% Mucinous: 9%; In combination with CA 125: sensitivity = 69%, specificity = 95%
Kallikrein 10	Luo (Clin Cancer Res) 2001, Luo (Clinica Chimica Acta) 2001[b], Luo 2003[a], Shvartsman 2003,	54–78%	90–100%	Sensitivity by stage: Stage I: 6%, Stage II: 18%, Stage III: 52%, Stage IV: 50% Sensitivity by histology: Serous: 47%, Endometrioid: 13%, Mucinous: 5% In combination with CA 125, sens = 73%; Sensitivity for stage I/II = 35%;
Kallikrein 11	Diamandis 2002	50%	In other cancers: 100% in lung and pancreatic cancers, 79% in colon cancer, 95% in thyroid cancer	

Table 1, continued

Category/Marker	References	Sensitivity	Specificity	Comments
MMP-2 (matrix metalloproteinase 2)	Schmalfeldt 2001	66%	100%	
Prostasin	Mok 2001[a]	51%	94%	In combination with CA125: sensitivity = 92%, specificity = 94%
Matriptase	Oberst 2002[a]	72%		Sensitivity by stage: Stage I: 81%, Stage II: 100%, Stage III: 58%; Sensitivity by histology: Serous: 61%, Mucinous: 75%, Endometrioid: 82%, Clear cell: 67%
Cathepsin L[a]	Nishida 1995[a]	80%	85%	
Alpha-1-antitrypsin	Kawai 1989	79%	89% in benign gynecologic conditions, 10% in pregnant women, 100% in healthy women	
Thrombin-antithrombin III, D-dimer	den Ouden M 1998[a]	90%	94% in benign tumors	
Cytokines, targets and acute phase reactants				
Immunosuppressive acidic protein	Castelli 1991[a,b], Lin 1994[a], Sawada 1983[a,b], Shimzu 1986, Yamashita 1986	70.4–93%	91–96%; 67–75% using benign tumors; 96% in normal women	Sensitivity by stage: Stage I: 12–100 Stage II: 28–100% Stage III: 53–100 Stage IV: 80% Sensitivity by histology: Serous: 75–83% Mucinous: 100% Clear cell: 83–100% Poorly differentiated: 66.7–100% Papillary: 100% Germ cell: 71.4–100%
MCSF (macrophage coloy-stimulating factor)	Suzuki 1998[a], van Haaften-Day 2001, Woolas 1993[a], Woolas 1995[a], Xu 1991	29–100%	76% in women presenting with pelvic mass; 75–93% in women with benign disease; 92–98% in healthy controls	Sensitivity by stage: Stage I: 31–87% Stage II: 63–75% Stage III: 37%, Stage IV: 41–100%; In combination with CA 125, sensitivity = 98%; In combination with CA 125 and OVX1, sensitivity = 57-85%, specificity = 58–83%
Interleukin 6	Tempfer (Gyn Onc) 1997[b]			Mean levels of IL-6 (pg/ml) and range: In normal controls = 0.5 (0–2) In ovarian cancer = 56 (0–2869)
SIL-2Ra (soluable interleukin-2 receptor alpha)	Hurteau 1995[a,b], Sedlaczek 2002[a]	95%	5–97%	In combination with CA125, sensitivity = 89–100%, spec = 27–91%; 92% stage I/II had elevated sIL-2R or CA 125, 67% had elevations of both; Sensitivity by histology: Serous: 85%, Endometrioid: 75%, Mucinous: 86%, Undifferentiated: 73%
Alpha-Haptoglobin	Ye (Clin Cancer Res) 2003[a]	64%	90%	In combination with CA 125, 91% sensitivity and 95% specificity

Table 1, continued

Category/Marker	References	Sensitivity	Specificity	Comments
Hormones and growth/inhibition factors				
Activin A	Menon 2000[a,b]	48%	15%	
Gonadotropin fragments	Cole (Can Res) 1988[a,b], Cole (Gyn Onc)1988[a]	73–83%	92%	
Inhibins A and B	Frias 1999[a,b], Menon 2000[a,b], Robertson 2002	15–59%	95%	Sensitivity by histology: Serous: 10–18%, Mucinous: 72–84%, Endometrioid: 31–54%, Clear cell: 22-44% Undifferentiated: 33% Granulosa cell: 100% Sensitivity by stage: Stage I/ II: 14% Stage III/ IV: 86% Note: Variation in sensitivity depends on type of assay used to measure Inhibin
Mesothelin/ megakaryocyte potentiating factor (MPF)	Scholler 1999	77%	100% in healthy controls and patients with non-neoplastic diseases	Stages III–IV can be detected
TGF-alpha (transforming growth factor)	Chien 1997[a]	33–71%	89% in healthy women, 72% in benign ovarian tumors	Sensitivity by stage: Stage I: 60% Stage II: 71% Stage III: 63% Stage IV: 50% Sensitivity varies by histology: Serous: 71%, Endometrioid: 70%, Mucinous: 33%
P110 epidermal growth factor receptor	Baron 1999[a] Baron 2003[a]	56%; 73% at ages 20–40, 61% at ages 41–60, 33% at ages 61–87	94% in healthy women of all ages; 94% at ages 20–40, 94% at ages 41–60, 93% at ages 61–80	Sensitivity by stage: Stage I/II: 34% Stage III/IV: 61%; Median levels (fmol/ml): Healthy women: 7177, range (114–31,465) Ovarian cancer: 463, range (undetectable – 82,436)
ErbB-2 (HER 2-Neu)	Hellstrom 2001[a], van Haaften-Day 1996	25–50%	83%	Sensitivity by stage: Stage I/II: $< 25\%$, Stage III/IV: 100%; In combination with EGFR, sensitivity $= 81\%$ (and 59% in borderline tumors), specificity $= 48\%$
Cytokeratins				
TPA (tissue polypeptide antigen)	Inoue 1992[a,b], Kudoh 1999[a], Sedlaczek 2002[a], Toftager-Larsen 1992[a], Tholander 1990[a,b]	25–83.3%	54.5–96% in benign tumors	In combination with CA 125 sens $= 79.6$–92.6%, spec $= 50$–72.7% (both positive), sens $= 59.3$–64.8%, spec $= 88.6$–97.7% Sensitivity by stage: Stage I: 50% Stage II: 78% Sensitivity by histology: Serous: 60–76% Endometrioid: 30–81% Mucinous: 35–86% Undifferentiated: 82%

Table 1, continued

Category/Marker	References	Sensitivity	Specificity	Comments
M3/M21	Hefler 1998, Tempfer (BJC) 1997[a,b]	57–78%	85–98%	Ovarian cancer patients with M3/M21 levels $<$ 45 U l-1 survive significantly longer than those with levels $>$ 45 U l-1; Mean serum levels (U/I) of M3/M21 and range: In healthy controls: 25.2 (7.7-5.1) In ovarian cancer: 52.4 (0.1-4,595)
Lipo-proteins				
LPA (lysophosphatidic acid)	Xu 1998	90–97.9%	89.6%	Sensitivity by stage: Stage I: 90% Stage II-IV: 100% Mean LPA level ([b]mol/L) and standard error: In healthy controls $= 0.6 \pm 0.19$ In ovarian cancer $= 8.6 \pm 1.45$
LSA (lipid-assocaited sialic acid)	Petru 1990	79%	63%	
Apolipoprotein (a)	Kuesel 1992[a]	81–89%	66–77%	Sensitivity by stage: Stage I/II: 84% Stage III/IV: 89%
Oncofetal proteins				
CEA	Engelen 2000[a], Inoue 1992[a,b], Kudoh 1999[a], Roman 1998[a] Tholander 1990[a,b], Tamakoshi 1996,	9–29%	87–97% in women with benign tumors 65% in women with ovarian cysts	CEA in combination w/ CA 125: Sensitivity = 87–94% Specificity = 80–87% Sensitivity by histology: Serous: 0–13.8% Mucinous: 33–80%, Endometrioid: 0–20% Clear cell = 8.7%; In borderline tumors, sensitivity = 9%
PLAP (placental-like alkaline phosphatase)	Ind 1997[a], Nozawa 1990, Tholander 1990[a,b], Toftager-Larsen 1992[a]	25–58%	77–100%; 100% in healthy women, In women with benign tumors = 68–94%	Sensitivity by stage: Stage I: 25–50%, Stage II: 42% Stage III: 47% Stage IV: 58%; Incombination with CA 125 (at least one positive), sens = 78–83%, spec = 57–71% (both tests positive), sens = 30–43%, spec = 98
Auto-antibodies				
P53	Gadducci 1996[a]	33%	93% in women with endometrial cancer	Sensitivity by stage: Stage I/II: 22%, Stage III: 31%, Stage IV: 50%
Ep-Cam	Kim 2003[a]	73%	81% in normal controls, 77% in benign ovarian disease	Mean auto-antibody levels: Normal controls = 0.09 (range 0.05, 0.13) Benign disease = 0.10 (range 0.06, 0.15) Ovarian cancer = 0.13 (range 0.08, 0.20)
Others				
Osteopontin	Kim 2002[a]	80–85%	80%	Sensitivity by stage: Stage I/II: 80%, Stage III/IV: 85% Mean level (ng/mL) and 95% CI:

Table 1, continued

Category/Marker	References	Sensitivity	Specificity	Comments
				In healthy: 147 (8–641) In benign: 254.4 (3–641) In ovarian cancer: 487 (315–751)
ALF (a-L-fucosidase)	Abdel-Aleem 1996[a], Beattie 1993	89%	98%	Mean ALF level (IU/ml) and standard deviation: In healthy controls = 114 ± 56, In familial cases = 159 ± 50, In sporadic cases = 124 ± 54
Galactosyltranserase	Udagawa 1998[b], Sichel 1994	46%	5% in benign ovarian tumors	Sensitivity by histology: Serous: 56%, Endometrioid: 50%;
LDH (lactate dehydrogenase)	Kudoh 1999[a], Schneider 1997[a]	60–87%;	93%	Sensitivity by histology: Serous: 57.1% Mucinous: 33%, Endometrioid: 50%, Clear cell: 35%; Sensitivity in peritoneal fluid (87%) is higher than in serum (60%)
Tetranectin (TN)	Hogdall 2000[a]	17%	94% in benign tumors	Sensitivity by stage: Stage I/II: 4%, Stage III/IV: 35%
EDN (RNAaseA)	Ye (AACR) 2003[a]	70–75%	80–94%; 80% for all subtypes, 94% for non-mucinous	Sensitivity by stage: Stage I/II: 71%, Stage III/IV: 75%
Protein patterns				
Cluster pattern algorithm	Petricoin 2002[a]	100%	95%	

[a]Indicates that pre-operative samples were used in the study.
[b]Indicates that CV is available in the study.

125 [58]. It is not known whether combining several kallikreins will improve sensitivity and specificity, but this seems unlikely since they appear to be upregulated as group based on their tight genetic clustering. Prostasin is another serine protease, whose gene was discovered to be upregulated in ovarian cancer. When combined with CA 125, its sensitivity to detect ovarian cancer was improved from 51% to 92% with a combined specificity of 94%, similar to what was observed for kallikrein 10 [62].

The matrix metalloproteinases (MMP's) are another family of proteases that may be useful in ovarian cancer screening or prognosis. Unlike the kallikreins, genes for the MMP's are not clustered in the same chromosomal region. MMP-2 was reported to have 66% sensitivity and 100% specificity in a study that needs confirmation [83]. Other proteases described for ovarian cancer include urokinase-type plaminogen activator [97], matriptase [70], and thrombin [16]. Cathepsin L is a cysteine protease. Its level in the serum was found to be significantly elevated in ovarian cancer but no sensitivity estimate was provided [68]. The same paper described a lower false positive rate for this marker compared to CA 125 or CA 72-4 but null correlations suggesting it might be complementary to these markers.

For several of the proteases, their complementary inhibitors have been identified and may also be useful for ovarian cancer screening. Matriptase and its inhibitor hepatocyte growth factor activator inhibitor 1, [70] and urokinase and its inhibitor, plasminogen activator inhibitor type I [97], are examples of complementary pairs. Another protease inhibitor, HE4 was identified through gene upregulation studies and may prove useful for ovarian cancer screening [37]. Its complementary protease has apparently not been identified. Finally, thrombin, its inhibitor, anti-thrombin III, as well as a degradation product D-Dimer are examples of a triad of protease, inhibitor, and degradation product which may all be applicable for ovarian cancer screening [16] and provide a useful paradigm for research on other sets of proteases, inhibitors, and cleavage products. Indeed, cleavage products of proteases may prove to be a large and important group of markers for ovarian and other types of cancers. It is very likely that many of the low molecular weight "peaks" being identified through mass spectrometry (see Proteomic Patterns below) represent cleavage products of unidentified proteases.

Based on their biochemical role, it would seem likely that the proteases would be active in invasion and metastasis, and these markers do appear to have higher sensitivity for advanced stages of disease. A potential

advantage of this category of markers based on their lower molecular weight is that urine might be a useful medium for screening. Indeed the MMPs have been found in urine for a number of cancers [66].

4. Cytokines, targets, and acute phase reactants

Arguably next in importance for ovarian cancer screening are the cytokines, which may prove to have good sensitivity for ovarian cancer, although it seems likely that their specificity may suffer from levels being elevated with other types of cancers as well as inflammatory conditions. Perhaps the best-known marker for ovarian cancer in this category is macrophage colony stimulating factor (MCSF), which was reported to have a sensitivity of 75 to 100% even for early stage disease and specificity of 92% to 98% in healthy controls [98,102,103]. Tumor necrosis factor (TNF)-alpha is a cytokine that inhibits growth by increasing apoptosis. Consequently, one might expect its levels to be reduced in individuals with cancer. However, investigators reported no difference in TNF-alpha levels between individuals with ovarian cancer and those with benign tumors [75]. Yet, a chemical relative, TNF-related apoptosis-inducing ligand was found to have a ten fold increase in ovarian cancer compared to normal ovarian epithelium, suggesting that increased levels may indicate the presence of ovarian cancer [55]. Interleukin (IL) 6 levels were significantly increased in serum and peritoneal fluid in ovarian cancer and correlated with stage of disease, although no sensitivity and specificity estimates provided [65,92]. Additional markers we include in this category are the soluble form of the IL receptors and acute phase reactant proteins. Soluble IL 2 receptor alpha had a sensitivity of 95% but very poor specificity improved by combining the marker with CA 125 [44]. The acute phase reactant protein alpha haptoglobin has also been identified as a potential marker for ovarian cancer. Interestingly, intact haptoglobin was identified years previously as a screening marker with modest sensitivity and specificity for multiple cancers. The fragment of haptoglobin alpha was rediscovered through mass spectrometry and found to have improved sensitivity and specificity when combined with CA-125. Finally, C-reactive protein levels were reported to be $\geqslant$ 50 mg/l in 20% stage I/II and 30% of stage III/IV ovarian cancers and to be associated with poorer survival [53].

5. Hormones, growth/inhibition factors

Based upon their role in normal ovarian physiology, epithelial tissue physiology, or tumor physiology, it would seem that a variety of hormones and growth or inhibition factors might be of value for ovarian cancer. However, few are of proven value for epithelial ovarian cancer screening, although several have been useful in immunohistochemical studies that correlate tumor staining properties with prognosis. As a key factor in ovarian physiology, the gonadotropins have been an important focus in animal studies but their measurement in serum does not appear useful in ovarian cancer screening. However, there has been some interest in urinary "gonadotropin fragments" with sensitivity ranging from 73 to 83% and specificity about 92% [15]. The inhibins are also key players in ovarian physiology and are representatives of a large group of proteins known as the transforming growth factor (TGF) beta superfamily, many of which have tumor suppressor or promoter functions. Although the inhibins have not shown particular value in the detection of epithelial ovarian cancer, they may prove useful for the diagnosis of granulosa cell tumors [11,76]. Serum TGF-alpha has also been examined for ovarian cancer with sensitivity estimates of 60–70% for early stage disease and specificity estimates of 89% for healthy controls [13]. Perhaps the current best performing marker in the growth factor category is Mesothelin, which was identified in gene upregulation studies, with a sensitivity of 77% (mostly late stage disease) and specificity of 100% [82]. In addition to these growth or inhibition factors, soluble forms of their receptors have also been studied as potential markers. The epidermal growth factor receptor, called p110, has good specificity at 94% in healthy controls, but its sensitivity declines with age. At ages 20–40, the p110 sensitivity is 73%, but it declines to 33% in older women [4].

Several markers in this family have been used in histochemical staining of tumors and might correlate with prognosis. Epidermal growth factor assessed by immunohistochemical staining of tumors was found to be only marginally predictive of ovarian cancer [72]; and no association between the EGF receptor and stage of disease was found [98]. Other tumor markers of potential interest for "typing" tumors based on their immunohistochemistry include p53 and HER 2 neu. The reported proportion of epithelial ovarian cancers that express p53 varies widely, ranging from 3 to almost 50% [59,78,98]. P53 expression is much lower at early stage than late stage disease [59,78]. P53 expression

also varies by histology with the highest proportion of p53 positive cases in the invasive serous group and the lowest in the mucinous group [78]. P53 expression is virtually absent borderline tumors, and no benign tumors stained positively for p53 expression [98]. HER-2 neu is found in less than 25% of newly diagnosed ovarian cancers [36].

6. Cytokeratins

Further related to epithelial cell biology, the cytokeratins are a group of intermediate filament proteins that compose the cytoskeleton of epithelial cells. Although individual cytokeratins may be relevant to ovarian cancer, more frequently mixtures of them have been studied. The best known of these is tissue (specific) polypeptide antigens (TPA or TPS). A wide range of sensitivities and specificities has been reported but the best performance has been achieved when this marker is combined with CA 125. In several studies, the reported sensitivity was between 79 and 93% and the reported specificity between 89 and 98% [47,84,94,95]. A mixture of two cytokeratins, known as M3/M21 has also been studied as a marker for ovarian cancer with poorer sensitivity but better specificity than TPA [35, 92].

7. Lipids and lipo-proteins

There was considerable excitement and interest in a lipid marker for ovarian cancer, lysophosphatidic acid (LPA) based on a 1998 report [114], which described a sensitivity of 97.9% and specificity of 89.6%. The requirement of this assay for quickly processed and frozen plasma appears to have affected enthusiasm for this marker although a search for more stable forms is underway. Apolipoprotein A has a reasonable sensitivity, ranging from 81–89%, but poor specificity (23–34%). Both sensitivity and specificity varied by age. At ages less than 49, sensitivity is high but specificity is poor. For ages greater or equal to 49, sensitivity decreases but specificity increases [115]. Not shown in Table 1 since serum levels have not been reported are two other apolioproteins, apolipoprotiens E and J (also known as clusterin). Both of these were found to be highly upregulated in large scale serial analysis of gene expression (SAGE) and may prove of value in ovarian cancer screening or prognosis [43].

8. Oncofetal proteins

Of the traditional oncofetal proteins, carcinogenic embroyonic antigen (CEA) and alpha-fetal protein (AFP), CEA has been the topic of several studies while AFP appears to have diagnostic value only for the germ cell tumors. CEA had very little sensitivity by itself for detecting epithelial ovarian cancer. Although not traditionally considered an oncofetal protein, placenta like alkaline phosphatsase (PLAP), was identified decades ago by similar techniques used to identify CEA and AFP; i.e. studies to identify antigens common to cancer and fetal tissue. PLAP has been the topic of several studies, but like CEA appears to have reasonable performance in screening only when combine with CA 125 with a sensitivity of 93% and a specificity of 83% [94].

9. Auto-antibodies

The neoplastic process is associated with the entry of a variety of cancer proteins into the circulation that may lead to auto-antibody production. Although a very small number of screening markers currently fit into this category, we think it is important to include this as a distinct and potentially important group of biomarkers for ovarian cancer. Antibodies to p53 have been evaluated but showed poor sensitivity and appear to be of little screening value [30,41]. Rare cases of cerebellar degeneration in association with ovarian cancer have been described and appear to represent a paraneoplastic response to auto-antibodies that react with Purkinje cells [38]. Based on the fact that the cerebellar symptoms often preceded a clinical diagnosis of ovarian cancer, it seems reasonable to speculate that auto-antibody formation could precede other clinical symptoms and thus be a potentially good marker for early stage disease [64]. Currently, the only marker studied for its potential as a screening tool is based upon auto-antibodies directed against the epithelial cell adhesion molecule (Ep-CAM). This study compared Ep-CAM auto-antibody levels in the sera of 26 healthy controls, 26 women with benign disease, and 26 ovarian cancer patients. Investigators report that the Ep-CAM auto-antibody screening showed a sensitivity of 73.1% and a specificity of 80.8% (95% CI = 65.7 to 95.9), while CA125 alone in the same set of sample provided a sensitivity of 86.5% and a specificity of 88.5%. The combination of Ep-CAM auto-antibody and CA125 gave a sensitivity of 90.4% and specificity of 92.3% [51].

10. Other markers

A variety of other markers not easily classified have been studied in ovarian cancer screening or prognosis. These include several markers identified through biochemical assays, multifunction proteins, and general measures of the degree of gycosylation. Enzymatic tests of potential value for ovarian cancer include alpha-fucosidase, galacotsyltransferase, and LDH [1,8, 54,81,86,96]. RNAase A is a biomarker initially assessed for other cancers but found to be elevated in the urine of ovarian cancer patients identified through mass spectrometric approaches [107]. Osteopontin is a multifunction protein identified through gene upregulation studies and found to have higher mean levels in sera from ovarian cancer subjects compared to that from normal women or those with benign disease [50]. Lipid – associated sialic acid provides a more global measure of sialic acid, an amino carbohydrate, that is an important component of tumor glycoproteins and does correlate with advanced ovarian and other types of cancer. However, this test lacks specificity since it may be associated with non-neoplastic conditions as well.

11. Proteomic patterns

We conclude our review of markers for ovarian cancer with the recent innovative approach of using mass spectrograph patterns of proteins or small molecules as the screening test [73]. This potential advance has been permitted by high throughput mass spectrometry combined with sophisticated biostatistical approaches to identify patterns in the output. When presented with a test set of sera (or other biologic specimens), the algorithm was able to find a pattern that distinguished cancer cases from controls with near perfect accuracy and that was reproducible when presented with specimens from a comparable set of cases and controls. However, as was illustrated in the article describing this approach, when presented with a novel set of alternate controls, the algorithm assigned a "new pattern" for most of these controls. Thus, the transferability of the algorithm to novel specimens as well as the ability to standardize the output from mass spectrometry appear to be current obstacles for adapting this approach to clinical care. Other studies using mass spectrometry have attempted to identify components of the predictive patterns – most of which appear to be in the low molecular weight range, which may make this an ideal tool for the discovery of urinary markers for ovarian cancer.

12. Combinations of markers

In reviewing Table 1 it is apparent that sensitivities and specificities were often provided for combinations of 2 or more markers (usually with CA 125 as one of these). Table 2 highlights these combinations for easier comparison. Some of the better performing combinations of markers include CA 125 and HMFG1/G2 with a sensitivity of 93% and a specificity of 93% [18] as well as CA 125 and CEA with a sensitivity of 93% and a specificity of 93% [94]. CA 125 with TAG72 and CA15-3 increase the specificity to 95% but sacrifice sensitivity [7]. We should note here that the traditional approach to combining multiple markers in most of these studies has been to use fixed cutoffs for each marker. Figure 1 illustrates the limitations of this approach in a simple two marker example using actual data on haptoglobin-alpha and CA 125 from the paper by Ye [106]. Using fixed cutoffs for both markers and declaring a positive screen to occur when either cutoff is exceeded and a negative screen to occur only when both values are below the cutoff, subjects within the box are declared negative and subjects outside the box are declared positive. This rule leads to 6 cases falsely declared negative and 18 controls falsely declared positive.

However a linear or logistic function of the two parameters, represented by the diagonal line, declares a negative screen to occur below the diagonal and a positive screen to occur above the diagonal. This rule leads to 8 cases falsely declared negative and 10 controls falsely declared positive; i.e. a substantial improvement in specificity with only a modest decrease in sensitivity. The problem becomes more complex when the distributions for the markers are not normal or when more than two markers are being examined. Logistic regression models for more than two variables or mixtures of multivariate normal distributions have been suggested. While a full discussion goes beyond the scope of this paper, the topic is considered in more detail elsewhere [33,87].

13. Discussion

To some extent, the markers identified in Table 1 reflect the dual evolution of cancer model systems and available biologic tools. Some of the earliest models relied upon experimentally-induced tumors in animals and used relatively simple gel and immune based tools to identify cancer antigens not found in adult tissue but

Table 2
Sensitivity and specificity of markers combinations

Marker combinations	References	Sensitivity (%)	Specificity (%)	Method of analysis
CA 125, TPS, sIL-2Ra	Sedlaczek 2002	100	72	Cutoffs
CA 125, HMFG1/G2	Dhokia 1986	95	93	Cutoffs
CA 125, CEA	Tholander 1990	93	83	Cutoffs
CA 125, Haptaglobin alpha	Ye 2003	91	95	Logistic Regression
CA 125, Ep-CAM	Kim 2003	90	92	Logistic Regression
CA 125, TN, OVX1, CASA	Hogdall 2000	89	66	Cutoffs
CA 125, TPA, PLAP	Toftager-Larsen 1992	85	59	Cutoffs
CA 125, OVX1, MCSF	Van Haaften-Day 2001	85	83	Cutoffs
CA 125, IAP	Castelli 1991	85	95	Cutoffs
CA 125, OVX1, LASA, CA15-3, CA72-4	Woolas 1995	83	84	Logistic Regression
CA 125, TAG72, CA15-3	Bast 1991	79	95	Cutoffs
CA 125, hK10	Luo 2003	73	90	Cutoffs
CA 125, CEA, STN	Inoue 1992	71	76	Cutoffs
CA 125, MCSF, OVX1	Woolas 1993	67	89	Cutoffs

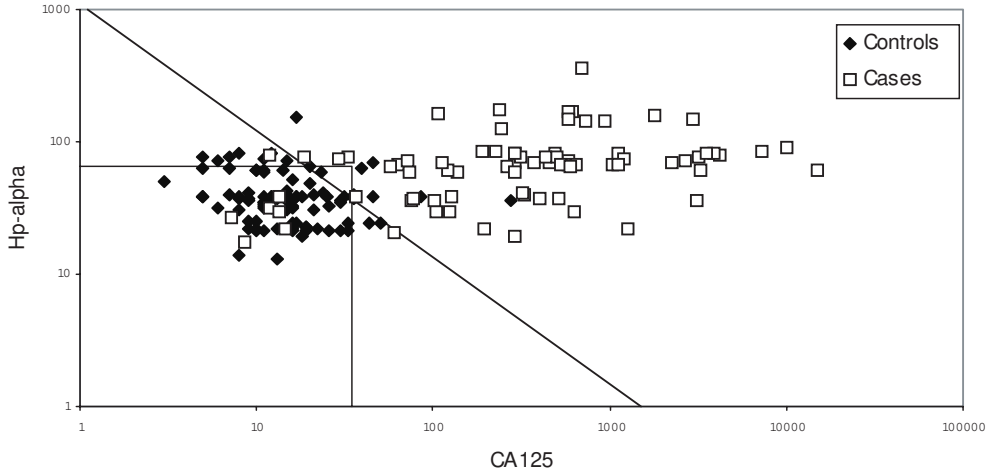

Fig. 1. Fixed cutoffs versus linear models in a study of two markers.

often found in fetal tissue from the same organ in the animal model. These led to identification of oncofetal proteins like CEA as some of the earliest cancer biomarkers. Since many early diagnostic tests in the general medical clinic relied upon enzymatic tests, it is not surprising that many of these found a role as early cancer diagnostic tests, including lactate dehydrogenase and acid phosphatase. With the evolution of mammalian cell culturing techniques including capacity for monoclonal anti-body production, the stage was set for a productive era of discovery of human cancer associated antigens, including CA 125 for ovarian cancer. Recognition of the role of tumor promoters and suppressors in cancer formation and the development of powerful new genetic techniques led to discovery of a number of genes up or downregulated in cancer. This in turn has allowed focus on their gene products as potential cancer markers. Most recently the development of precise and high throughput mass spectrography has shifted the focus to proteomic patterns rather than specific markers. These methods are well suited for the discovery of entirely new biomarkers since the technique begins with no assumptions about the disease model and allows for post-translational modifications to be detected.

Most of the biologic models leading to marker development have focused on cancer cell cultures and invasive disease. Thus, it is not surprising that many of the markers identified so far are indicative of cell death, tissue remodeling or invasion, inflammatory response elements, or systemic response markers. These types of markers would seem to be most appropriate in late stage disease as appears to be the case for most of the markers in Table 1. Perhaps, new paradigms will be necessary for detection of markers suited for detection of early stage disease. Methods better suited to

detect trace concentrations associated with low tumor load, early genetic changes, or subtle changes related to tumor surveillance cells or the immune system are needed. With regard to the latter, we postulate that the largely unexplored area of auto-antibody formation to tumor products might prove useful for early stage disease. Another technical limitation is our ability to describe and quantitate glycosylation. Aberrant glycosylation has been known as a feature of cancer for many years, especially apparent for the epithelial sialomucins. Thus, advances may await newer techniques to quantitatively and qualitatively describe glycosylation and take this into consideration in marker assays.

Other limitations are also clearly apparent in reviewing the studies cited in Table 1, including those that affect the reliability of the sensitivity and specificity estimates. Precision of the estimates of sensitivity and specificity will be greater for larger versus smaller study populations; but the effect produced by the use of different populations in studies poses a problem less able to be predicted. Case groups may have different mixtures of disease stages and histologic types and grades that affect overall estimates of sensitivity. In addition, although some groups were explicit in stating that case specimens were limited to those collected pre-operatively, not all groups stated this allowing for the possibility that some specimens were collected in the immediate post-operative period. Few studies have used pre-diagnostic sera months or years before clinical diagnosis. Obviously, the types of controls selected will greatly influence the estimated specificity of a particular marker or combination of markers. Inclusion of surgical controls that have other gynecological conditions such as fibroids, endometriosis, or benign ovarian tumors will lead to lower specificity compared to non-surgical controls. In terms of relevance to general population screening, specificity estimates based upon healthy controls are best. Description of the case or controls populations sometimes did not include the proportions that were pre- and postmenopausal. CA 125 is known to vary by menopausal status and it is likely that a number of other markers will vary as well. A final limitation is that assay performance was seldom addressed in detail by including estimates of inter- and intra-assay coefficients of variation. Poor assay performance could seriously compromise the reproducibility of sensitivity and specificity estimates.

Conceding these limitations, we still had the goal in preparing this review to identify individual markers or combination of markers with the best performance. Individual serum markers measured by immunoassay that appeared to perform best (if we require a specificity of at least 90% and sensitivity of 75% or greater) included the following: CA 125, CA 15-3 and its variants, MAM-6, kallikrein 10, thrombin-antithrombin III, mesothelin, IAP, MCSF, SIL-2RA, and TPA. Combinations of markers achieving these objectives include CA 125 in combination with HMFG1/2, in combination with TAG 72 and CA 15-3, or in combination with IAP, or haptoglobin alpha. Clearly, high specificity is demanded if expensive or invasive diagnostics tests are required to follow-up on a positive screening test; and the 90% specificity required above may not be good enough. Singling out some markers with specificities above 98% (while maintaining sensitivities of at least 60%) revealed some of the following markers TAG-72, kallikrein 6 or 10, MMP-2, and Mesothelin. As a suggestion for future combinations to examine, it would be reasonable to combine one or more marker from each category in Table 1 in hopes that complementary markers could be identified to improve sensitivity while selecting additional markers which have excellent specificity. One of the most important characteristics needed for future ovarian cancer markers is high sensitivity at early stages.

Our belief that there may already be a very satisfactory set of markers that exist for ovarian cancer must be tempered by the likelihood that this panel may be positive for benign ovarian diseases and other cancers. Thus, follow-up for a positive test may require a battery of imaging studies. Imaging studies that reveal a complex ovarian mass will likely lead to surgery and in some cases will reveal a benign tumor. We would argue this should not be considered a failure of screening. A woman with a 10 cm complex ovarian tumor requires surgery not only because of the mass's potential to be cancerous but also because of its potential to undergo torsion or rupture producing a surgical emergency. A "positive panel" for ovarian cancer not associated with an obvious ovarian mass will require additional imaging or screening studies that are already recommended on a routine basis including mammogram, colonoscopy, or chest x-ray.

In conclusion, as the search for new markers for ovarian cancer continues, it is important to re-assess what has been already learned in the past three or four decades of previous research on ovarian cancer. Good and likely reliable markers already exist that simply need to be combined together in more convenient assay platforms. New research would be worthwhile and should focus on underdeveloped areas such as autoantibodies to cancer proteins or further descriptors of

glycosylation. Mass spectrometry approaches may reveal complex patterns of cleavage products that provide unique cancer signatures; but there will still be a need for identifying these products to determine their role in cancer pathogenesis or progression. Since the focus of research has largely been on serum markers, attention should also be given to the urinary markers, which may be ideally suited to investigation using the mass spectrometric approaches.

References

[1] H. Abdel-Aleem, A. Ahmed, A.M. Sabra, M. Zakhari, M. Soliman and H. Hamed, Serum alpha L-fucosidase enzyme activity in ovarian and other female genital tract tumors, *Int J Gynecol Obstet* **55** (1996), 273–279.

[2] G.L. Adonkis, E. Paraskevaidis, S. Tsiga, K. Seferiadis and D.E. Lolis, A combined approach for the early detection of ovarian cancer in asymptomatic women, *Eur J Obstet Gynecol Reprod Biol* **65** (1996), 221–225.

[3] A. Anisowicz, G. Sotiropoulou, G. Stenman, S. Mok and R. Sager, A novel protease homolog differentially expressed in breast and ovarian cancer, *Molec Med* **2** (1996), 624–636.

[4] A.T. Baron, E.M. Cora, J.M. Lafky, C.H. Boardman, M.C. Buenafe, A. Rademaker, D. Liu, D.A. Fishman, K.C. Podratz and N.J. Maihle, Soluble epidermal growth factor receptor (sEGFR/sErbB1) as potential risk, screening, and diagnostic serum biomarker of epithelial ovarian cancer, *Cancer Epidemiology, Biomarkers & Prevention* **12** (2003), 103–113.

[5] A.T. Baron, J.M. Lafky, C.H. Boardman, S. Balaubramaniam, V.J. Suman, K.C. Podratz and N.J. Maihle, Serum sErbB1 and epidermal growth factor levels as tumor biomarkers in women with stage III or IV epithelial ovarian cancer, *Cancer Epidemio Biomarkers Prev* **8** (1999), 129–137.

[6] R.C. Bast, T.L. Klug, E. St. John, E. Jenison, J.M. Niloff, H. Lazarus, R.S. Berkowitz, T. Leavitt, C.T. Griffiths, L. Parker, V.R. Zurawski and R.C. Knapp, A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer, *N Engl J Med* **309** (1983), 883–887.

[7] R.C. Bast, S. Knauf, A. Epenetos, B. Dhokia, L. Daly, M. Tanner, J. Soper, W. Creasman, S. Gall and R.C. Knapp, Coordinate elevation of serum markers in ovarian cancer but not in benign disease, *Cancer* **68** (1991), 1758–1763.

[8] G.J. Beattie, J.E. Roulston, D.M. Eccles, J.M. Richardson, J. Fisken and R.C.F. Leonard, Alpha-fucosidase as a marker of genetic deletion in ovarian carcinoma, *Ann Clin Biochem* **30** (1993), 207–208.

[9] A. Ben-Arie, Z. Hagay, H. Ben-Hur, M. Open and R. Dgani, Elevated serum alkaline phosphatase may enable early diagnosis of ovarian cancer, *Eur J Obstet Gynecol* **86** (1999), 69–71.

[10] M. Bhattacharya, S.K. Chatterjee and J.J. Barlow, UridineS-diphosphat-galacotse: glycoprotein galactosyl-transferase activity in the ovarian cancer patient, *Cance Res* **36** (1976), 2096–2101.

[11] H.G. Burger, P.J. Fuller, S. Chu, P. Mamers, A. Drummond, B. Susil, P. Neva and D.M. Robertson, The inhibins and ovarian cancer, *Mol Cell Endocrinol* **180**(1–2) (2001), 145–148.

[12] M. Castelli, F. Battaglia, G. Scambia, P. Benedetti Panici, G.Ferrandina, A.M. Mileo, S. Mancuso and U. Ferrini, Immunosuppressive acidic protein and CA 125 levels in patients with ovarian cancer, *Oncology* **48** (1991), 13–17.

[13] C.H. Chien, C.C. Huang, Y.H. Lin, J. Shen and S.N. Chow, Detection of serum transforming growth factor-alpha in patients of primary epithelial ovdarian cancers by enzyme immunoassay, *Gynecol Oncol* **66** (1997), 405–410.

[14] L.A. Cole, Y.X. Wang, M. Elliott, M. Latif, J.T. Chambers, S.K. Chambers and P.E. Schwartz, Urinary human chorionic gonadotropin free beta-subunit and beta-core fragment: a new marker of gynecological cancers, *Cancer Res* **48** (1988), 1356–1360.

[15] L.A. Cole, P.E. Schwartz and Y.X. Wang, Urinary gonadotropin fragments in cancers of the female reproductive system. I. Sensitivity and specificity, comparison with other markers, *Gynecol Oncol* **31** (1988), 82–90.

[16] M. Den Ouden, J. Ubachs, J. Stoot and Versch, Thrombin-antithrombin III and D-dimer plasma levels in patients with benign or malignant ovarian tumors, *Scand J Clin Lab Invest* **58** (1998), 555–560.

[17] H.W. De Bruijn, K.A. ten Hoor, H. Boonstra, J. Marrink, M. Krans and J.G. Aalders, 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* **14** (1993), 105–115.

[18] B. Dhokia, P.A. Canney, D. Pectasides, A.J. Munro, M. Moore, P.M. Wilkinson, C. Self and A.A. Epenetos, A new immunoassay using monoclonal antibodies HMFG1 and HMFG2 together with an existing marker CA125 for the serological detection and management of epithelial ovarian cancer, *Br J Cancer* **54** (1986), 891–895.

[19] E.P. Diamandis, A. Okui, S. Mitsui, L.-Y. Luo, A. Soosaipillai, L. Grass, T. Nakamura, D.J.C. Howarth and N. Yamaguchi, Human Kallikrein 11: A new biomarker of prostate and ovarian carcinoma, *Cancer Research* **62** (2002), 295–300.

[20] E.P. Diamandis, G.M. Yousef, A.R. Soosaipillai and P. Bunting, Human Kallikrein 6 (zyme/protease M/neurosin): a new serum biomarker of ovarian carcinoma, *Clin Biochem* **33** (2000), 579–583.

[21] E.P. Diamandis, A. Scorilas, S. Fracchioli, M. van Gramberen, H. de Bruijn, Henrik, A. Soosaipillai, L. Grass, G.M. Yousef, U. Stenman, M. Massobrio, A.G. van der Zee, I. Vergote and D. Katsaros, Human kallikrein 6 (hK6): A new potential serum biomarker for diagnosis and prognosis of ovarian carcinoma, *J Clin Oncol* **21** (2003), 1035–1043.

[22] S.M. Ellerbroek, D.A. Fishman, A.S. Kearns, L.M. Bafetti and M.S. Stack, Ovarian carcinoma regulation of matrix metalloproteinase-2 and membrane type 1 matrix metalloproteinase through beta 1 integrin, *Cancer Res* **59** (1999), 1635–1641.

[23] M.J.A. Engelen, H.W.A. de Bruijn, H. Hollema, K.A. ten Hoor, P.H.B. Willemse, J.G. Aalder and A.G.J. van der Zee, Serum CA 125, carcinoembryonic antigen, and CA 19-9 as tumor markers in borderline ovarian tumors, *Gynecol Oncol* **78** (2000), 16–20.

[24] H. Feng, Ghazizadeh, H. Konishi and T. Araki, Expression of MUC1 and MUC2 gene products in human ovarian carcinomas, *Jpn J Clin Oncol* **32** (2002), 525–529.

[25] X. Filella, R. Molina, J. Jo, J.L. Bedini, J. Joseph and A.M. Ballesta, Tumor associated glycoprotein-72 (TAG-72) levels in patients with non-malignant and malignant disease, *Bull Cancer* **79** (1992), 271–277.

[26] D.A. Fishman, Y. Liu, S.M. Ellerbroek and M.S. Stack, Lysophosphatidic acid promotes matrix metalloproteinas (MMP) activation and MMP-dependent invasion in ovarian cancer cells, *Cancer Res* **61** (2001), 3194–3199.

[27] J. Fisken, J.E. Roulston, C. Sturgeon, R.A. Badley, I. Jonrup, L. Aspinall and R.C.F. Leonard, The value of the human milk fat globule membrane antigen HMFG2 in epithelial ovarian cancer monitoring: comparison with CA125, *Br J Cancer* **67** (1993), 1065–1070.

[28] A.E. Frias, H. Li, G.L. Keeney, K.C. Podratz and T.K. Woodruff, Preoperative serum level of inhibin A is an independent prognositc factor for the survival of postmenopausal women with epithelial ovarian carcinoma, *Cancer* **85** (1999), 465–471.

[29] A. Gadducci, M. Ferdeghini, C. Prontera, L. Moretti, L. Pellagatta, R. Bianchi and P. Fioretti, CA 195: a new monoclonal antibody-defined Le^a blood group-related tumor marker in patients with epithelial ovarian cancer, *Journal of Nucl Biol Med* **35** (1991), 33–37.

[30] A. Gadducci, M. Ferdeghini, F. Buttitta, A. Fanucchi, C. Annicchiarico, C. Prontera, G. Bevilacqua and A.R. Genazzani, Preoperative serum antibodies against the p53 protein in patients with ovarian and endometrial cancer, *Anticancer Res* **16**(6B) (1996), 3519–3523.

[31] F. Guadagni, C. Marth, A.G. Zeimet, P. Ferroni, A. Spila, R. Abbolito, M. Roselli, J.W. Greiner and J. Schlom, Evaluation of tumor-associated glycoprotein-72 and CA125 serum markers in patients with gynecologic diseases, *Am J Obstet Gynecol* **171**(5) (1994), 1183–1191.

[32] B.L. Harlow, N.S. Weiss and E. Holmes, Plasma Alpha-L-Fucosidase activity and the risk of borderline epithelial ovarian tumors, *Cancer Res* **50** (1990), 4702–4703.

[33] T. Hastie and R. Tibshirani, Discrimnant analysis by Gaussian mixtures, *Journal of the Royal Statistical Society Series B – Methodological* **58**(1) (1996), 155–176.

[34] L. Hefler, C. Tempfer, H. Heinzl, G. Haeusler, P. Steindl, C. Schneeberger, G. Gitsch and C. Kainz, M3/M21 serum levels in women with adnexal masses and inflammatory diseases, *Int J Cancer* **79** (1998), 434–438.

[35] L. Hefler, C. Tempfer, G. Hausler, H. Heinzl and C. Kainz, Cytokeratin tumor markers in ovarian carcinoma: tissue polypeptide specific antigen (TPS) and M3/M21, *Wiener Klinische Wochenschrift* **110**(18) (1998), 635–641.

[36] I. Hellstrom, G. Goodman, J. Pullman, Y. Yang and K.E. Hellstrom, Overexpression of HER-2 in ovarian carcinomas, *Cancer Research* **61**(6) (2001), 2420–2423.

[37] I. Hellstrom, J. Raycraft, M. Hayden-Ledbetter, J.A. Ledbetter, M. Schummer, M. McIntosh, C. Drescher, N. Urban and K.E. Hellstrom, The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma, *Cancer Res* **63**(13) (2003), 3695–3700.

[38] D.J. Hetzel, C.R. Stanhope, B.P. o'Neill and V.A. Lennon, Gynecologic cancer in patients with subacute cerebellar degeneration predicted by anti-Purkinje cell antibodies an dlimited in metastatic volume, *Mayo Clinic Proceedings* **65**(12) (1990), 1558–1563.

[39] J. Hilkens, V. Kroezen, J.M.G. Bonfrer, M. De Jong-Bakker and P.F. Bruning, MAM-6 antigen, a new serum marker for breast cancer monitoring, *Cancer Res* **46** (1986), 2582–2587.

[40] E.V. Hogdall, C.K. Hogdall, S. Tingulstad, B. Hagen, K. Nustad, F.-J. Xu and R.C. Bast, Predictive values of tumor markers tetranectin, OVX1, CASA, and CA125 in patients with a pelvic mass, *Int J Cancer* **89** (2000), 519–523.

[41] E.V. Hogdall, C.K. Hogdall, J. Blaakaer, N.H. Heegaard, E. Glud, L. Christensen, J.E. Bock, B. Norgaard-Pedersen, A. Wiik and S.K. Kjaer, P53 autoantibodies in sera from Danish ovarian cancer patients and their correlation with clinical data and prognosis, *APMIS* **110**(7–8) (2002), 545–553.

[42] C.D. Hough, K.R. Cho, A.B. Zonderman, D.R. Schwartz and P.J. Morin, Coordinately up-regulated genes in ovarian cancer, *Cancer Res* **61**(10) (2001), 3869–3876.

[43] C.D. Hough, C.A. Sherman-Baust, E.S. Pizer, F.J. Montz, D.D. IM, N.B. Rosenshein, G.J. Riggins and P.J. Morin, Large-scale serial analysis of gene expression reveals genes differentially expressed in ovarian cancer, *Cancer Res* **60**(22) (2000), 6281–6287.

[44] J.A. Hurteau, R.P. Woolas, I.J. Jacobs, D.C. Oram, C.C. Kurman, L.A. Rubin, D.L. Nelson, A. Berchuck, R.C. Bast Jr and G.B. Mills, Soluble interleukin-2 receptor alpha is elevated in sera of patients with benign ovarian neoplasms and epithelial ovarian cancer, *Cancer* **76** (1995), 1615–1620.

[45] T. Ind, R. Iles, J. Shepherd and T. Chard, Serum concentrations of cancer antigen 125, placental alkaline phosphatase, cancer-associated serum antigen and free beta human chorionic gonadotrophin as prognostic markers for epithelial ovarian cancer, *Br J Obstet Gynecol* **104** (1997), 1024–1029.

[46] M. Inoue, H. Ogawa, K. Nakanishi, O. Tanizawa, K. Karino and J. Endo, Clinical value of sialyl Tn antigen in patients with gynecologic tumors, *Obstetrics & Gynecology* **75**(6) (1990), 1032–1036.

[47] M. Inoue, M. Fujita, A. Nakazawa, H. Ogawa, O. Tanizawa, Sialyl-Tn and X.I. Silyl-Lewis, CA 19-9, CA 125, carinoembryonic antigen, and tissue polypeptide antigen in differentiating ovarian cancer from benigh tumors, *Obstet Gynecol* **79** (1992), 434–440.

[48] I.J. Jacobs, D.H. Oram and R.C. Bast, Strategies for improving the specificity of screening for ovarian cancer with tumor-associated antigens CA 125, CA 15-3, and TAG 72.3, *Obstet Gynecol* **80** (1992), 396–399.

[49] M. Kawai, N. Iida, S. Inagaki, H. Yamashita, H. Imaizumi, M. Kakihara and Y. Arii, Acute phase protein (Alpha 1 AT) as a tumor marker in patients with ovarian cancer, *Gan No Rinsho* **35**(7) (1989), 799–803.

[50] J.H. Kim, S. Skates, T. Uede, K. Wong, J.O. Schorge, C.M. Feltmate, R.S. Berkowitz, D.W. Cramer and S.C. Mok, Osteopontin as a potential diagnostic biomarker for ovarian cancer, *JAMA* **287**(13) (2002), 167–169.

[51] J. Kim, D. Herelyn and W. Kwong-kwok et al., Identification of Ep-CAM autoantibody in patients with ovarian cancer, *Clinical Cancer Res* **9** (2003), 4728–4791.

[52] S. Knauf, Clinical evaluation of ovarian tumor antigen NB/70K: monoclonal antibody assays for distinguishing ovarian cancer from other gynecological disease, *Am J Obstet Gynecol* **158** (1988), 1067–1072.

[53] J. Kodama, Y. Miyagi, N. Seki, K. Tokumo, M. Yoshinouchi, Y. Kobashi, H. Okuda and T. Kudo, Serum c-reactive protein as a prognostic factor in patients with epithelial ovarian cancer, *European Journal of Obstetrics & Gynecology and Reproductive Biology* **82** (1999), 107–110.

[54] K. Kudoh, Y. Kikuchi, T. Kita, T. Tode, M. Takano, J. Hirata, Y. Mano, K. Yamamoto and I. Nagata, Preoperative determination of several serum tumor markers in patients with primary epithelial ovarian carcinoma, *Gynecol Obstet Invest* **47** (1999), 52–57.

[55] J.M. Lancaster, B.C. Blanchette, B. Calingaert, R. Whitaker, J. Schildkraut, J. Marks and A. Berchuck, High expression of tumor necrosis factor-related apoptosis-inducing ligand is

associated with favorable ovarian cancer survival, *Clinical Cancer Research* **9**(2) (2003), 762–767.

[56] L. Luo, D. Katsaros, A. Scorilas, S. Fracchioli, R. Piccinno, I.A. Rigaul de la Longrais, D.J.C. Howarth and E.P. Diamandis, Prognostic value of human kallikrein 10 expression in epithelia ovarian carcinoma, *Clin Cancer Res* **7** (2001), 2372–2379.

[57] L. Luo, P. Bunting, A. Scorilas and E.P. Diamandis, Human kallikrein 10: a novel tumor marker for ovarian cancer? *Clinica Chimica Acta* **306** (2001), 111–118.

[58] L. Luo, D. Katsaros, A. Scorolas, S. Fraccjop;o, R. Bellino, M. van Gramberen, H. de Bruijn, U. Stenman, M. Massobrio, A.G.J. van der Zee, I. Vergote and E.P. Diamandis, The serum concentration of human kallikrein 10 represents a novel biomarker for ovarian cancer diagnosis and prognosis, *Cancer Res* **63** (2003), 807–811.

[59] H. Meden, D. Marx, W. Rath, M. Kron, A. Fattahi-Meibodi, B. Hinney, W. Kul and A. Schauer, Overexpression of the oncogene c-erb B2 in primary ovarian cancer: evaluation of the prognositc value in a Cox proportional hazards multiple regression, *Int J Gynecol Pathol* **13**(1) (1994), 45–53.

[60] U. Menon, S.C. Riley, J. Thomas, C. Bose, A. Dawnay, L.W. Evans, N.P. Groome and I.J. Jacobs, Serum inhibin, activin and follistatin in postmenopausal women with epithelial ovarian carcinoma, *Br J Obstet Gynaecol* **107** (2000), 1069–1074.

[61] U. Menon and I.J. Jacobs, Recent developments in ovarian cancer screening, *Curr Opin Obstet Gynecol* **12** (2000), 39–42.

[62] S.C. Mok, J. Chao, S. Skates, K. Wong, G.K. Yiu, M.G. Muto, R.S. Berkowitz and D.W. Cramer, Prostasin, a potential serum marker for ovarian cancer: identification through microarray technology, *J Natl Cancer Inst* **93** (2001), 1458–1464.

[63] R. Molina, B. Ojeda, X. Filella, G. Borras, J. Jo, E. Mas, J.J. Lopez and A. Ballesta, A prospective study of tumor markers CA 125 and CA19.9 in patients with epithelial ovarian carcinomas, *Tumor Biol* **13** (1992), 278–286.

[64] J.W. Moll, S.C. Henzen-Logmans, T.A. Splinter, M.E. van der Burg and C.J. Vecht, Diagnostic value of anti-neuronal antibodies for paraneoplastic disorders of the nervous system, *Journal of Neurology, Neurosurgery & Psychiatry* **53**(11) (1990), 940–943.

[65] M.M. Moradi, L.F. Carson, B. Weinberg, A.F. Haney, L.B. Twiggs and S. Ramakrishnan, Serum and ascitic fluid levels of interleukin-1, interleukin-6, and tumor necrosis factor-alpha in patients with ovarian epithelial cancer, *Cancer* **72** (1993), 2433–2440.

[66] M.A. Moses, D. Wiederschain, K.R. Loughlin, D. Zurakowski, C.C. Lamb and M.R. Freeman, Increased incidence of matrix metalloproteinases in urine of cancer patients, *Cancer Research* **58** (1998), 1395–1399.

[67] Y. Negishi, H. Iwabuchi, H. Sakunaga, M. Sakamoto, K. Okabe, H. Sato and G. Asano, Serum and tissue measurements of CA72-4 in ovarian cancer patients, *Gynecologic Oncology* **48**(2) (Feb 1993), 148–154.

[68] Y. Nishida, K. Kohno, T. Kawamata, K. Morimitsu, M. Kuwano and I. Miyakawa, Increased cathepsin L levels in serum in some patients with ovarian cancer: comparison with CA125 and CA72-4, *Gynecologic Oncology* **56** (1995), 357–361.

[69] S. Nozawa, Y. Udagawa, H. Ohkura, Y. Negishi, K. Akiya, N. Inaba, H. Takamizawa, E. Kimura and Y. Terashima, Serum placental alkaline phosphatase (PLAP) in gynecologic malignancies – with special reference to the combination of PLAP and CA54/61, *Clinica Chimica Acta* **186**(2) (1990), 275–284.

[70] M.D. Oberst, M.D. Johnson, R.B. Dickson, C.Y. Lin, B. Singh, M. Stewart, A. Williams, A. al-Nafussi, J.F. Smyth, H. Gabra and G.C. Sellar, Expression of the serine protease matriptase and its inhibitor HAI-1 in epithelial ovarian cancer: correlation with clinical outcome and tumor clinicopathological parameters, *Clinical Cancer Research* **8**(4) (April 2002), 1101–1107.

[71] O.J. Owens and R.E. Leake, Growth factor content in normal and benign ovarian tumours, *Eur J Obstet Gynecol Reprod Biol* **47**(3) (1992), 223–228.

[72] O.J. Owens, C. Stewart, I. Brown and R.E. Leake, Epidermal growth factor receptors (EGFR) in human ovarian cancer, *Br J Cancer* **64**(5) (1991), 907–910.

[73] E.F. Petricoin, A.M. Ardekani, B.A. Hitt, P.J. Levine, V.A. Fusaro, S.M. Steinberg, G.B. Mills, C. Simone, D.A. Fishman, E.C. Kohn and L.A. Liotta, Use of proteomic patterns in serum to identify ovarian cancer, *Lancet* **359** (2002), 572–577.

[74] E. Petru, B.U. Sevin, H.E. Averette, O.R. Koechli, J.P. Perras and S. Hilsenbeck, Comparison of three tumor markers – CA 125, lipid-associated sialic acid (LSA), and NB/70 – in monitoring ovarian cancer, *Gynecol Oncol* **38** (1990), 181–186.

[75] R. Punnonen, K. Teisala, T. Kuppala, B. Bennett and J. Punnonen, Cytokine production profiles in the peritoneal fluids of patients with malignant or benign gynecological tumors, *Cancer* **83**(4) (1998), 788–796.

[76] D.M. Robertson, T. Stephenson, E. Pryusers, H.G. Burger, P. McCloud, A. Tsigos, N. Groome, P. Mamers, J. McNeilage, T. Jobling and D. Healy, Inhibins/activins as diagnostic markers for ovarian cancer, *Molecular and Cellular Endocrinology* **191** (2002), 97–103.

[77] L.D. Roman, L.I. Muderspach, A.F. Burnett and C.P. Morrow, Carcinoembryonic antigen in women with isolated pelvic masses. Clinical utility? *Journal of Reproductive Medicine* **43**(5) (1998), 403–407.

[78] R.A. Sagarra, L.A. Andrade, E.Z. Martinez, G.A. Pintos, K.L. Syrjanen and S.F. Derchain, P53 and Bcl-2 as prognostic predictors in epithelial ovarian cancer, *Int J Gynecol Cancer* **12** (2002), 720–727.

[79] M. Sawada, Y. Okudaira, Y. Matsui and Y. Shimzu, Immunosuppressive acidic protein in patients with ovarian cancer, *Cancer* **52** (1983), 2081–2085.

[80] G. Scambia, P. Benedetti, G. Panici, L. Baiocchi, L. Perrone, S. Greggi, P. DiRoberto and S. Mancuso, CA 15-3 serum levels in ovarian cancer, *Oncology* **45** (1988), 263–267.

[81] D. Schneider, R. Halperin, R. Langer, I. Bukovsky and A. Herman, Peritoneal fluid lactate dehydrogenase in ovarian cancer, *Gynecologic Oncology* **66** (1997), 399–404.

[82] N. Scholler, N. Fu, Y. Yang, Z. Ye, G.E. Goodman, K.E. Hellstrom and I. Hellstrom, Soluble member(s) of the mesothelin/megakaryocyte potentiating factor family are detectable in sera from patients with ovarian carcinoma, *Proc Natl Acad Sci* **96** (1999), 11531–11536.

[83] B. Schmalfeldt, D. Prechtel, K. Harting, K. Spathe, S. Rutke, E. Konik, R. Fridman, U. Berger, M. Schmitt, W. Kuhn and E. Lengyel, Increased expression of matrix metalloproteinases (MMP-2), MMP-9, and the urokinase-type plaminogen activator is associated with progression from benign to advanced ovarian cancer, *Clin Cancer Res* **7** (2001), 2396–2404.

[84] P. Sedlaczek, I. Frydecka, M. Gabrys, A. van Dalen, R. Einarsson and A. Harlozinska, Comparative analsyis of CA 125, tissue polypeptide specific antigen, and soluble interleukin-2 receptor alpha levels in sera, cyst, and ascitic fluids from patients with ovarian carcinoma, *Cancer* **95** (2002), 1886–1893.

[85] H.S. Shvartsman, K.H. Lu, J. Lee, J. Lillie, M.T. Deavers, S. Clifford, J.K. Wolf, G.B. Mills, R.C. Bast, D.M. Gershenson and R. Schmandt, Overexpression of kallikrein 10 in epithelial ovarian carcinomas, *Gynecol Oncol* **90** (2003), 44–50.

[86] F. Sichel, V. Salaun, E. Bar, P. Gauduchon, J.-P. Malas, J. Goussard and J.-Y. Talaer, Biological markers and ovarian carcinomas: galactosyltransferase, CA 125, isoenzymes of amylase and alkaline phosphatase, *Clinica Chimica Acta* **227** (1994), 87–96.

[87] S.J. Skates, F. Xu, Y.H. Yu, K. Sjovall, N. Einhorn, Y. Chang, R.C. Bast and R.C. Knapp, Toward an optimal algorithm for ovarian cancer screening with longitudinal tumor markers, *Cancer* **76**(10 Suppl) (1995), 2004–2010.

[88] M. Suzuki, I. Sekiguchi and T. Tamanda, Clinical evaluation of tumor-associated mucin-type glycoprotein CA 54/61 in ovarian cancers: comparison with CA 125, *Obstet Gynecol* **76** (1990), 422–427.

[89] M. Suzuki, H. Kobayaski, M. Ohwada, T. Terao and I. Sato, Macrophage colony-stimulating factor as a marker for malignant germ cell tumors of the ovary, *Gynecol Oncol* **68** (1998), 35–37.

[90] Y. Tamada, S. Iida, D. Aoki, S. Nozawa and T. Irimura, Carbohydrate epitopes and mucin expressed by 17 human ovarian carcinoma cell lines, *Oncol Res* **11** (1999), 233–241.

[91] K. Tamakoshi, F. Kikkawa, K. Shibata, K. Tomoda, N.H. Obata, F. Wakahara, Y. Tokuhashi, H. Ishikawa, M. Kawai and Y. Tomoda, Clinical value of CA125, CA19-9, CEA, CA72-4, and TPA in borderline ovarian tumor, *Gynecol Oncol* **62** (1996), 67–72.

[92] C. Tempfer, L. Hefler, G. Haeusler, G. Sliutz, E. Hanzal, A. Reinthaller and C.H. Kainz, Serum M3/M21 in ovarian cancer patients, *British Journal of Cancer* **76**(10) (1997), 1387–1389.

[93] C. Temper, H. Zeisler, Sliutz, H. Guenther, E. Hanzal and C. Kainz, Serum evaluation of interleukin 6 in ovarian cancer patients, *Gyn Oncol* **66** (1997), 27–30.

[94] B. Tholander, A. Taube, A. Lindgren, O. Sjoberg, U. Stendahl, A. Kiviranta, K. Hallman, L. Holm, E. Weiner and L. Tamsen, Pretreatment serum levels of CA-125, carcinoembryonic antigen, tissue polypeptide antigen, and placental alkaline phospatase, in patients with ovarian carcinoma, borderline tumors, or benign adnexal masses: relevance for differential diagnosis, *Gynecol Oncol* **39** (1990), 16–25.

[95] K. Toftager-Larsen, U. Hording, A. Dreisler, S. Daugaard, B. Lund, J. Bock, F. Lundvall, K. Frederiksen and B. Norgaard-Pedersen, CA-125, placental alkaline phospatase and tissue polypeptide antigen as preoperative serum markers in ovarian carcinoma, *Gynecol Obstet Invest* **33** (1992), 177–182.

[96] Y. Udagawa, D. Aoki, K. Ito, T. Uejima, M. Uemura and S. Nozawa, Clinical characteristics of a newly developed ovarian tumour marker, galactosyltransferase associated with tumor (GAT), *European Journal of Cancer* **34**(4) (1998), 489–495.

[97] M.E. van der Burg, S.C. Henzen-Logmans, E.M. Berns, W.L. van Putten, J.G. Kli and J.A. Foekens, Expression of urokinase-type plasminogen activator (uPA) and its inhibitor PAI-1 in benign, borderline, malignant primary and metastatic ovarian tumors, *Int J Cancer* **69**(6) (1996), 475–479.

[98] C. Van Haaften-Day, P. Russell, C.M. Boyer, B.J. Kerns, J.R. Wiener, D.N. Jensen, R.C. Bast and N.F. Hacker, Expression of cell regulatory proteins in ovarian borderline tumors, *Cancer* **77**(10) (1996), 2092–2098.

[99] R.H. Verheijen, S. von Mensdorff-Pouilly, G.J. van Kamp and P. Kenemans, CA 125: fundamental and clinical aspects, *Seminars in Cancer Biology* **9** (1999), 117–124.

[100] M.C. Wang, L.A. Valenzuela, G.P. Murphy and T.M. Chu, Purification of a human prostate specific antigen, *Journal of Urology* **167**(3) (2002), 1226–1230.

[101] R.P. Woolas, F. Xu, I.J. Jacobs, Y. Yu, L. Daly, Clarke-Pearson, D.H. Oram and R.C. Bast, Elevation of multiple serum markers in patients with stage I ovarian cancer, *JNCI* **85** (1993), 1748–1751.

[102] R.P. Woolas, M.R. Conaway, F. Xu, I.J. Jacobs, Y. Yu, L. Daly, A. Prys Davies, K. O'Briant, A. Berchuck, J.T. Soper, D.L. Clarke-Pearson, G. Rodriguez, D.H. Oram and R.C. Bast, Combinations of multiple serum markers are superior to individual assays for discriminating malignant from benign pelvic masses, *Gynecologic Oncology* **59** (1995), 111–116.

[103] F.-J. Xu, S. Ramakrishnan, L. Daly, J.T. Soper, A. Berchuck, D. Clarke-Pearson and R.C. Bast, Increased serum levels of macrophage colony-stimulating factor in ovarian cancer, *Am J Obstet Gynecol* **165** (1991), 1356–1362.

[104] F.-J. Xu, Y.-H. Yu, L. Daly, K. DeSombre, L. Anselmino, G.M. Hass, A. Berchuck, J.T. Soper, D.L. Clarke-Pearson, C. Boyer, L.J. Layfield and R.C. Bast, OVX1 radioimmunoassay complements CA-125 for predicting the presence of residual ovarian carcinoma at second-look surgical surveillance procedures, *J Clin Oncol* **11** (1993), 1506–1510.

[105] K. Yamashita, H. Hayashi, K. Mure, M. Ishikawa and T. Shimizu, Serum immunosuppressive substance in patients with gynecologic malignancies and in pregnant women, *Cancer* **57** (1986), 69–74.

[106] B. Ye, D.W. Cramer, S.J. Skates, S.P. Gygi, V. Pratomo, L. Fu, N.K. Horick, L.J. Licklider, J.O. Schorge, R.S. Berkowitz and S.C. Mok, Haptoglobin-a subunit as potential serum biomarker in ovarian cancer: Identification and characterization using proteomic profiling and mass spectrometry, *Clinical Cancer Research* **9** (2003), 2904–2911.

[107] B. Ye, H.F. Rosenberg, L. Fu, S. Skates, R.S. Berkowitz, S.C. Mok and D. Cramer, Identification and validation of Eosinophil-derived neurotoxin as a candidate urinary biomarker for early stage of ovarian cancer by SELDI-MS profiling. AACR Proceedings of 94th Annual Meeting Cancer Research Vol 44, Washington DC, p 1366, Cancer Research 2003.

[108] B.W. Yin and K.O. Lloyd, Molecular cloning of the CA125 ovarian cancer antigen, *J Biolog Chem* **276** (2001), 27371–27375.

[109] G.M. Yousef and E.P. Diamondis, Kallikreins, steroid hormones, and ovarian cancer: Is there a link? *Minerva Endocrinolo* **27** (2002), 157–166.

[110] G.M. Yousef and E.P. Diamandis, Expanded human tissue kallikrein family – A novel panel of cancer biomarkers, *Tumor Biol* **23** (2002), 185–192.

[111] A.G. Zeimet, F. Guadagni, C. Marth, T. Amato, E. Muller-Holzner, O. Huter, G. Daxenbichler and O. Dapunt, Vaue of the TAG-72 (CA72-4) tumor marker in primary diagnosis of ovarian carcinoma. A comparison with the established CA-125 marker, *Geburtshilfe und Frauenheilkunde* **55** (1995), 195–199.

[112] V.R. Zurawski, K. Sjovall, D.A. Schoenfeld, S.F. Broderick, P. Hall, R.C. Bast, G. Eklund, B. Mattsson, R.J. Connor, P.C. Prorok, R.C. Knapp and N. Einhorn, Prospective evaluation of serum CA 125 levels in a normal population, phase I: specificities of single and serial determinations in testing for ovarian cancer, *Gynecol Oncol* **36** (1990), 299–305.

[113] I.J. Jacobs, S.J. Skates, N. MacDonald, U. Menon, A.N. Rosenthal, A.P. Davies, R. Woolas, A.R. Jeyarajah, K. Sibley, D.G. Lowe, D.H. Oram, Screening for ovarian cancer: a pilot randomised controlled trial, *Lancet* **353** (1999), 1207–1210.

[114] Y. Xu, Z. Shen, D.W. Wiper, M. Wu, R. Morton, P. Elson, A. Kennedy, W. Alexander, J. Belinson, M. Markman, G. Casey, Lysophosphatidic acid as a potential biomarker for ovarian cancer and other gynecologic cancers, *JAMA* **280** (1998), 719–723.

[115] A. Kuesel, T. Kroft, M. Prefontaine, I.C.P. Smith, Lipoprotein(a) and CA125 levels in the plasma of patients with benign and malignant ovarian disease, *Int J Cancer* **52** (1992), 341–346.

[116] C. Van Haaften-Day, Y. Shen, F. Xu, Y. Yu, A. Berchuck, L.J. Havrilesky, H.W.A. de Bruijn, A.G.J. van der Zee, R.C. Bast, N.F. Hacker, OVX1, macrophage-colony stimulating factor, and CA-125-II as tumor markers for epithelial ovarian carcinoma, *Cancer* **92** (2001), 2837–2844.

[117] M.A. McGuckin, G.T. Layton, M.J. Bailey, T. Hurst, S.K. Khoo, B.G. Ward, Evaluation of two new assays for tumor-associated antigens, CASA and OSA, found in the serum of patients with epithelial ovarian carcinoma – comparison with CA125, *Gynecol Oncol* **37** (1990), 165–171.

[118] Y. Lin, S. Chow, C. Chen, R. Chen, S. Huang, G. Huang, Evaluation of immunosuppresive acidic protein in human ovarian cancer, *J Formos Med Assoc* **93** (1994), 776–780.

[119] Y. Shimzu, E. Akagaki, K. Hirota, M. Kono, S. Miura, Y. Okudaira, K. Kurachi, Significance of immunosuppressive acidic protein in the diagnosis and follow-up of patients with ovarian cancer, in particular, as a marker for chemotherapeutic effects, *Nippon Sanka Fujinka Gakkai Zasshi* **38** (1986), 554–560.