The advancement of biomarker-based diagnostic tools for ovarian, breast, and pancreatic cancer through the use of urine as an analytical biofluid

Brian M. Nolen[1,2] and **Anna E. Lokshin**[1,2,3,4]

[1]University of Pittsburgh Cancer Institute, Pittsburgh, PA - USA

[2]Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA - USA

[3]Department of Pathology, School of Medicine, University of Pittsburgh, Pittsburgh, PA - USA

[4]Department of Obstetrics and Gynecology, School of Medicine, University of Pittsburgh, Pittsburgh, PA - USA

Abstract

Despite considerable advancements, the development of effective cancer screening tools based on serum biomarker measurements has thus far failed to achieve a meaningful clinical impact. The incremental progress observed over the course of serum biomarker development suggests that further refinements based on novel approaches may yet result in a breakthrough. The use of urine as an analytical biofluid for biomarker development may represent such an approach. The unique characteristics of urine including a high level of stability, ease of sampling, and an inactive and low-complexity testing matrix offer several potential advantages over the use of serum. A number of recent reports have demonstrated the utility of urine in the identification of novel cancer biomarkers and also the improved performance of biomarkers previously evaluated in serum. In this review, advancements related to the use of urine biomarkers within the settings of ovarian, breast, and pancreatic cancer are presented and discussed. Findings regarding the identification of specific urine biomarkers for each disease are highlighted along with comparative analyses of urine and serum biomarkers as diagnostic tools.

INTRODUCTION

Efforts to identify and validate biomarkers present in the bodily fluids of ovarian cancer patients are ongoing. Investigators hope to utilize these findings in the development of minimally invasive tests to predict tumorigenesis, disease recurrence, or treatment response.

Address for correspondence: Anna E. Lokshin University of Pittsburgh Cancer Institute Hillman Cancer Center, Suite 1.19d 5117 Centre Avenue Pittsburgh, PA 15213, USA lokshina@pitt.edu.

The bulk of this work has focused on blood, given its systemic exposure and extensive availability through tissue banks. The analysis of blood, through the use of either serum or plasma, carries with it several inherent limitations which have hindered the development of clinically useful biomarker assays. Foremost among these limitations is the relatively high level and complex nature of the protein repertoire found in blood. Components of the blood matrix, including clotting and other serological factors, carrier proteins, immunoregulatory proteins, and active enzymes, among others, all have the capacity to interefere with biomarker measurements. The clotting process itself, employed during the preparation of serum, has been shown to involve enzymatic activity which results in the cleavage of unrelated proteins of interest (1, 2). The invasive nature of blood testing also limits accessibility to repeated measurements and presents a risk of infection to both the patient and healthcare professionals, along with the added cost of minimizing this risk.

Recently, urine has been proposed as an alternative biofluid for analytical biomarker studies on the basis that the systemic nature of such testing might be preserved while several of the limitations inherent to blood testing could be eliminated. Urine is available in larger quantities than blood through less invasive means, allowing for repeated measurements aimed at patient surveillance or establishment of assay reproducibility. The urinary proteome is proposed to contain over 100,000 peptides, with 5000 of those present at high frequency (3), and studies have shown that this proteome is stable for hours at room temperature, days at 4°C, and years at −20°C (4). The urinary proteome is a direct product of renal filtration and consists of low-molecular-weight, soluble peptides which are highly amenable to proteomic analysis and may represent disease-specific cleavage processes (5). Renal filtration also results in a less complex matrix than that of blood, containing fewer factors known to interfere with biomarker assays (6). The use of urine as a diagnostic biofluid does present unique challenges including a high variability in protein concentrations due to differences in fluid intake. However, this barrier has been overcome successfully through normalization based on levels of creatinine or other common urinary peptides (7, 8). What remains in the development of urine-based analytical platforms is evidence that systemic disease-specific biomarkers are released into this biological compartment in a manner which can be reliably measured.

Traditionally, investigations focused on urinary biomarkers have been limited to those related to disorders of the urogenital system, although it is estimated that only 70% of the urinary proteome originates from the kidneys or urinary tract, with the remaining 30% resulting from the glomerular filtration of blood plasma (9). Urine therefore can be considered a systemic biofluid with expanded clinical applications. Such applications may include the early detection of various human malignancies, including those originating in tissues beyond the genitourinary system. Here, recent findings regarding the development of urine biomarkers aimed at the detection of ovarian, breast, and pancreatic cancer are reviewed. Findings regarding specific urine biomarkers are presented within each disease setting along with a discussion of the comparative performance of urine, serum, and combination biomarker panels.

OVARIAN CANCER

A number of significant findings have been reported through the analysis of urine obtained from ovarian cancer patients. Several early reports characterized the use of urinary gonadotropic peptide (UGP) as a general biomarker of gynecological malignancy (10–12). The combination of UGP and serum CA 125 proved particularly useful in the diagnosis of ovarian serous carcinomas, providing a sensitivity/specificity ratio of 86%/89% (11). More recently, several other biomarkers including HE4 (13), mesothelin (14), Bcl-2 (15), and angiostatin (16) were found to be differentially present in the urine of ovarian cancer patients and controls. In their respective studies, urinary HE4 was found to discriminate ovarian cancer patients from controls at a level similar to that of serum HE4, while urinary mesothelin outperformed its serum counterpart. Urine levels of angiostatin and Bcl-2 were associated with both early- and late-stage ovarian cancer in comparison to healthy and benign controls. Both markers were elevated in ovarian cancer patients regardless of tumor grade, stage, size, histological subtype, creatinine levels, age, or menopausal status, and displayed complementation with serum CA 125 measurements. Proteomic-based studies performed by several independent research groups have identified a number of additional urinary biomarkers and biomarker panels offering diagnostic potential for ovarian cancer (6, 17–20). Notable among these findings are a 3-biomarker panel which, in combination with CA 125, could discriminate malignant from benign pelvic masses with an area under the receiver-operating characteristic (ROC) curve (AUC) of 0.96 (19), and the combination of glycosylated eosinophil-derived neurotoxin and C-terminal osteopontin (OPN) fragments which provided a sensitivity of 94% at a specificity of 72% for early-stage ovarian cancer compared with benign controls (6).

Our group has previously reported on the use of serum biomarker panels for the discrimination of ovarian cancer patients from healthy controls and women with benign pelvic masses (21, 22). In order to evaluate the comparative performance of urine and serum biomarkers in these diagnostic settings, we analyzed levels of 15 biomarkers found to be significant in the previous studies in urine obtained from 108 patients diagnosed with ovarian cancer, 118 women with benign pelvic conditions, and 72 healthy controls (Tab. I). Fourteen of these biomarkers including HE4, CYFRA 21-1, EGFR, ErbB-2, IL-2R, sICAM-1, CEA, Eotaxin-1, sVCAM-1, CA 15-3, tPAI-1, CA 125, MMP-9, and MPO were detectable in urine, with CA 72-4 being the lone undetectable marker. The diagnostic performance of each urine biomarker was compared to our previously reported findings in serum (Fig. 1). In a ROC analysis of cancer patients and healthy controls, several urine biomarkers outperformed their serum counterparts from our previous study. Notably, this list included CEA, HE4, and sVCAM-1, 3 of the 4 markers included in the highest performing panel from that study. In the same comparison, CA 125 provided a significantly higher level of discrimination in serum compared to urine (data not shown). In the comparison of ovarian cancer patients and benign controls, sVCAM-1 and HE4 performed significantly better in urine (Fig. 1), while EGFR was most effective in serum (data not shown). The performance of multimarker panels in the discrimination of the ovarian cancer and control groups was evaluated using the previously described Metropolis-Monte Carlo (MMC) algorithm (22) (Fig. 2). A urine biomarker panel comprised of HE4, CEA, and CYFRA 21-1 was identified

as the optimal model for the classification of ovarian cancer cases and healthy controls. This panel significantly outperformed serum CA 125 measured in the same experimental group, and the addition of serum CA 125 to the urine biomarker panel resulted in a significant improvement in sensitivity. For the classification of benign versus malignant pelvic disease, HE4 alone measured in urine provided the highest diagnostic value, exceeding serum CA 125 and any combination of urine biomarkers. The combination of urine HE4 and serum CA 125 provided greater diagnostic utility. Overall, these results demonstrated that certain urine biomarkers and multimarker panels are capable of outperforming similar serum-based tests for diagnostic purposes and the combined use of urine and serum biomarker testing may provide a superior means of patient classification.

The minimally acceptable positive predictive value of 10% (1 case identified for every 10 individuals tested) required for effective ovarian cancer screening necessitates diagnostic tools which provide a high level of sensitivity and specificity (23, 24). Currently used tools such as CA 125 testing in blood and imaging procedures such as transvaginal ultrasound have failed to perform to this standard (25). Although many additional blood-based biomarkers for ovarian cancer have been identified and evaluated, little progress has been achieved in the development of diagnostic tests. Our preliminary study demonstrates that several previously identified serum biomarkers of ovarian cancer provide greater levels of diagnostic utility when evaluated in urine, and suggests that urine biomarker panels may provide levels of sensitivity and specificity for the discrimination of ovarian cancer patients from healthy controls approaching those required for routine screening. Several recent reports investigating the efficacy of biomarker panels within this setting have identified the combination of CA 125 and HE4 as an effective diagnostic tool capable of discriminating benign from malignant pelvic masses with high sensitivity and specificity (21, 26). This combination later showed efficacy in a prospective study (27) and was subsequently incorporated into a scoring model termed the Risk of Ovarian Malignancy Algorithm (ROMA) (28). Our results indicate that the incorporation of urine HE4 into this model may further improve the diagnostic efficacy.

BREAST CANCER

Effective screening has the potential to significantly ease the considerable public health burden imposed by breast cancer, which carries a 5-year survival rate of 97% when the disease is localized at diagnosis (29). However, screening efforts have been hampered by the limited effectiveness of mammography, which demonstrates an overall sensitivity of 75% (30), ranging from 54–58% in women younger than 40 years to 81–90% in postmenopausal women (31–33). A number of groups have investigated the use of circulating tumor markers as screening tools in breast cancer, but a clinically useful test has yet to emerge. Numerous serum tumor markers have been described for breast cancer, including members of the MUC family of mucin-associated glycoproteins (e.g., CA 15-3, BR 27.29, MCA, CA 549, CA 125, CA 72-4, and CEA), oncoproteins (e.g., HER-2/c-erbB-2), cytokines and growth factors (e.g., HGF, IL-6, and VEGF), and cytokeratins (e.g., TPA, TPS, and CYFRA 21-1) (34–42). Unfortunately, the diagnostic utility of all presently known serum tumor markers has been hampered by low sensitivity for early-stage disease and an overall lack of specificity.

In the past several years, the use of urine in studies aimed at the identification and development of breast cancer biomarkers has resulted in a number of diverse reports. Urine metabolite profiling through the use of a variety of techniques and strategies has produced several notable findings. Slupsky et al (20) utilized nuclear magnetic resonance spectroscopy to identify urine metabolite patterns specifically associated with early-stage breast and ovarian cancer. Elsewhere, 2 groups demonstrated the usefulness of metabolite panels consisting of 5 and 4 biomarkers, respectively, in the classification of breast cancer cases from controls (43, 44). The latter study identified their metabolite panel on the basis of genome-wide expression signatures. A separate investigation identified a panel of 31 nucleosides in urine which could discriminate breast cancer patients from controls with a sensitivity of 88% at a specificity of 90% (45). Melatonin, particularly the 6-sulfoxymelatonin derivative, has received significant attention as a urine biomarker of breast cancer. This biomarker has been shown to be useful in patient discrimination in both premenopausal (46) and postmenopausal (47, 48) women. Several other urine biomarkers, including N1,N12-diacetylspermine, ADAM-12, MMP-9, and several phytoestrogens have been evaluated with encouraging results, including the ability to outperform current serum biomarkers of breast cancer, CA 15-3 and CEA (49–51).

Our group recently reported on our analysis of serum proteins in healthy postmenopausal women and women diagnosed with breast cancer and benign breast lesions (52). Our bioinformatic approach identified a 3-biomarker panel consisting of MIF, MMP-9, and MPO that was able to distinguish healthy women from breast cancer patients with an AUC of 0.82 (sensitivity 60% at specificity 90%); however, benign and malignant cases were indistinguishable. More recently, we have conducted an analysis of the same proteins in urines obtained from 74 postmenopausal patients with early stage breast cancer and 90 age-matched healthy controls (Tab. I). On an individual basis, 4 biomarkers including ACTH, MPO, TSH, and FSH demonstrated significantly higher diagnostic capacities in urine in comparison to serum (Fig. 3). A multivariate analysis of the urine biomarker data identified a 3-biomarker panel consisting of FSH, MPO, and osteocalcin (OC) which provided a sensitivity of 86% at a specificity of 91% in the training set and a sensitivity of 75% at 93% specificity in a blinded validation set (Fig. 4), a 15% improvement in sensitivity over the serum results. Based on these results and the collective work of others it would appear that the advancement of biomarker-based diagnostics within the setting of breast cancer may rely more heavily on urine than serum going forward.

PANCREATIC CANCER

The devastatingly high mortality rate associated with pancreatic cancer indicates unmet areas of need in all aspects of the clinical management of this disease. One such area includes the development of effective screening tools to aid in early diagnosis. The benefits of improved screening are clear as the overall 5-year survival rises from <5% to 20–30% for patients with small, resectable, early-stage cancers (53–56). Current screening practices rely on the use of expensive and invasive procedures such as computed tomography (CT), magnetic resonance imaging (MRI), and endoscopic ultrasound (EUS). In addition to the expense and invasiveness inherent to these procedures, they are also associated with limited sensitivity and a high degree of overuse in response to vague symptoms such as abdominal

pain. A biomarker-based test that could complement or replace the use of CT or MRI, resulting in the more efficient referral of patients for invasive EUS, would offer considerable clinical value to physicians. CA 19-9, the most frequently studied biomarker for pancreatic cancer, had a median sensitivity of 79% (70–90%) and a median specificity of 82% (68–91%) in symptomatic patients (57). A number of other serum biomarkers including TPA/TPS, MIC-1, IGFBP-1, haptoglobin, SAA, TIMP-1, OPN, HE4, and NGAL have been evaluated, but none of these have proven to be superior to CA 19-9 (58–64). Several groups have also reported on the performance of multimarker combinations of these markers (65–68), including several reports involving the use of combined protein and nucleic acid panels (69–71). These multiplexed biomarker approaches have demonstrated improved sensitivity and specificity for the detection of pancreatic cancer. Multiplexed biomarker panels have also proven successful in the discrimination of benign from premalignant pancreatic lesions (72, 73).

The low prevalence and vague symptomatic presentation of pancreatic cancer dictate that a high level of performance will be required for any biomarker-based screening test to achieve clinical implementation. While the search for additional informative biomarkers is ongoing, the use of urine as an alternative to serum for biomarker analysis may result in advancements in the near future. Relatively few studies have been conducted to date regarding the use of urine biomarkers in the early diagnosis of pancreatic cancer. In one early report, UGP, a biomarker implicated in several malignant settings, was evaluated in patients with cancers of digestive organs, where it was found to be elevated in 61.5% of pancreatic cancers (74). More recent reports such as that by Weeks et al (75) suggest that urine may be a useful medium for biomarker analysis. Using proteomic methods the authors identified numerous altered peptides in patients diagnosed with pancreatic ductal adenocarcinoma (PDAC) in comparison with control individuals. Szajda et al (76, 77) in a series of reports identified several enzymes involved in carbohydrate metabolism as potential urine biomarkers of pancreatic cancer. Urine diacetylspermine was identified as a novel tumor marker for pancreatobiliary carcinoma with a sensitivity/specificity level similar to that of serum CA 19-9 (78).

We performed an analysis of 20 pancreatic-cancer-related biomarkers in sera and urines obtained from 55 patients diagnosed with PDAC and 44 patients diagnosed with a spectrum of benign pancreatic diseases (Tab. I). Our biomarker panel consisted of ApoA1, OC, osteoprotegrin (OPG), OPN, ACTH, insulin, PTH, leptin, MPO, CRP, SAA, sICAM-1, transglutaminase II, TIMP 1–4, CA 19-9, CA 125, and CEA. The performance of each biomarker in urine and serum was evaluated by ROC analysis (Fig. 5). On the basis of AUC 95% confidence intervals, CA 19-9 was the only marker which differed significantly, with a higher performance in serum. Among the remaining non-significant markers, 10 demonstrated higher AUC values in urine while 8 were higher in serum. Transglutaminase II was not detectable in urine. The mixed performance of this biomarker set in urine and serum led us to hypothesize that the development of diagnostic biomarker panels might benefit most from the combined use of urine and serum factors. In order to broaden our approach, urine samples were tested for an additional 198 biomarkers. A set of urines obtained from 45 healthy control individuals was included in this analysis. Multivariate analysis utilizing the MMC algorithm demonstrated that a urine 3-biomarker panel in combination with serum

CA 19-9 outperformed the best serum biomarker panel for the discrimination of PDAC from benign disease (Fig. 6). This panel did not outperform a serum biomarker panel consisting of CA 19-9, CEA, and TIMP-1, identified in our recent report (68), in terms of sensitivity (72% vs 76%) at 90% specificity. However, our previous study utilized a much larger patient cohort and candidate biomarker array. For the discrimination of patients with PDAC from healthy controls, a urine biomarker panel consisting of human growth factor (HGF) and stem cell factor (SCF) performed optimally with a sensitivity of 93% at a specificity of 90%. This panel did outperform the serum biomarker panel consisting of CA 19-9, sICAM-1, and OPG, which provided a sensitivity/specificity ratio of 88%/90% in our previous report (68). Thus, similar to our observation regarding ovarian cancer, the combined use of urine and serum biomarkers may provide the most robust means of improvement for the development of novel screening tools.

CONCLUSIONS

Although the incremental advancement of therapeutic agents and strategies has led to significant progress in cancer care over the course of the last decade, the accurate and timely detection and diagnosis of malignancy remains the single most important prognostic factor for nearly all affected individuals. Dramatic improvements in survival stemming from early detection are well described for each of the human cancers discussed in this review; however, each disease presents a unique set of challenges which has hampered or precluded the implementation of widespread screening. Despite the availability of useful biomarkers such as CA 125 and CA 19-9, the low prevalence associated with both ovarian and pancreatic cancer represents a significant obstacle which researchers and clinicians have yet to overcome. The high cost, morbidity, and patient anxiety associated with the diagnostic procedures employed within the settings of ovarian and pancreatic cancer, including both surgical and invasive imaging procedures, render the high level of false positive results generated through suboptimal screening practices unacceptable. While the increased prevalence of breast cancer allows for more permissive screening requirements, the disappointing performance of currently used methods such as mammography, the high cost of adjunct imaging modalities, and the current lack of useful biomarkers have limited the efficacy of screening strategies. Several avenues exist for the improvement and optimization of biomarker-based diagnostic tools. These include the development of multimodal screening strategies which incorporate biomarker testing and imaging; the discovery and development of novel specific biomarkers; and the use of alternative analytical biofluids displaying advantageous characteristics for biomarker development. The expanded use of urine in biomarker investigations offers the potential to spark improvement within each of these avenues and propel biomarker-based screening tools into broad clinical use.

The use of urine in biomarker analyses raises questions regarding the processing and filtration of serum proteins, particularly those of high molecular weight. This issue is well illustrated through our observations concerning CA 125, which did not productively contribute to diagnostic panel development when measured in urine. A plausible explanation for our observations stems from the considerable size of the CA 125 glycoprotein, estimated at 3–5 MDa, and the estimated molecular weight cutoff associated with glomerular filtration, 30–50 kDa. CA 125 was detectable in urine, suggesting that fragments of the molecule do

indeed pass through the glomerulus in a form which can be recognized by the immunoassay; however, the observed results indicate that cleavage processes responsible for such fragmentation are not reliable indicators of serum CA 125 levels. Several other biomarkers included in this investigation were also relatively large, with a molecular weight greater than 100 kDa, including CA 15-3, sEGFR, CEA, sVCAM-1 and MPO. A recent study examining the mechanisms of glomerular filtration concluded that in addition to molecular size, additional factors such as molecular conformation, charge, and deformability account for the ability of an individual molecule to be filtered (79). The authors of that study demonstrate that molecules as large as 350–500 kDa are rapidly cleared intact through the glomerulus. Such a phenomenon may indeed play a role in the detection of the protein biomarkers listed above, but it is also likely that the observed urine biomarker levels represent specific proteolytic cleavage processes. The latter notion suggests that observed increases or decreases in serum biomarker levels in cancer patients relative to controls may not persist in urine. We observed exactly that for CA 125, EGFR, MMP-9, MPO, and sVCAM-1 in our pancreatic cancer study described above.

The advancement of biomarker studies in urine will also require a detailed assessment of sampling strategies and methods of data normalization. The use of spot urines, or urines arbitrarily collected at the time of physician visit, represents a practical approach to urine collection, although this strategy also presents the disadvantages of variation in biomarker measurements resulting from dilution due to fluid intake, sample volume, and varying rates of urine production. As a result, urine biomarker levels are dependent not only on the rate of biomarker production, processing, filtration, and excretion, but also on the urinary flow rate. Normalization based on urine creatinine (UCr), a waste product of muscle metabolism which is excreted in urine via the glomerulus at relatively constant rates, is commonplace in urine biomarker studies (80, 81). However, the appropriateness of creatinine normalization in the study of urine biomarkers of cancer has not been examined in detail. Recent studies have demonstrated that UCr excretion rates vary significantly across individuals in general, across demographic and ethnic populations, and within individuals as a function of time and health status (80, 82–85). Thus, whenever the excretion rate of a particular biomarker of interest does not share a linear relationship with that of UCr, normalization based on UCr levels may confound biomarker measurements (80, 82, 86, 87). UCr excretion rates have also been observed to vary in response to gender, age, dietary protein levels, chronic inflammation, physical activity, and illness (88–93). Given that, within the setting of cancer, many of the factors described above are likely to coalesce, the relevance of UCr normalization in cancer biomarker studies should be further examined. In our studies, UCr normalization was performed and individual biomarker levels were evaluated both as absolute measurements and as ratios based on UCr levels. In general, markers which differed significantly between the case and control groups based on absolute biomarker levels maintained that significance when normalized data were analyzed. Biomarkers displaying the largest magnitude differences between groups, and thus those selected for diagnostic panel development, were impacted the least by creatinine normalization. Going forward, it may become apparent that the stringent performance requirements imposed upon biomarker-based screening tools, which necessitate the selection of highly altered and specific protein factors, will mitigate the need for normalization based on UCr.

The study of serum biomarkers associated with the spectrum of human malignancies has had a profound impact on the overall clinical management of patients, particularly in the areas of screening, diagnosis, prognosis, and monitoring. However, while much has been accomplished, much more remains to be done. Serum biomarker-based tests have thus far failed to overcome the specific challenges posed within each malignant setting and achieve their promised clinical goals. Further advancement will require the development of novel methods and strategies. The use of urine in biomarker analyses has the potential to exceed and complement the results observed in serum. Further development and validation of biomarker testing in both serum and urine should help maximize the clinical impact of these tools.

List of Abbreviations

ACTH	adrenocorticotropic hormone
AUC	area under the curve
CEA	carcinoembryonic antigen
CRP	C-reactive protein
CT	computed tomography
EGFR	epidermal growth factor receptor
EUS	endoscopic ultrasound
FSH	follicle stimulating hormone
HE4	human epididymus protein 4
HGF	human growth factor
IGFBP	insulin-like growth factor binding protein
MCA	mucin-like carcinoma-associated antigen
MIC-1	macrophage inhibitrory cytokine 1
MIF	macrophage inhibitory factor
MMC	Metropolis-Monte Carlo
MMP	matrix metalloproteinase
MPO	myeloperoxidase
MRI	magnetic resonance imaging
NGAL	neutrophil gelatinase-associated lipocalin
OC	osteocalcin
OPG	osteoprotegrin
OPN	osteopontin
PDAC	pancreatic ductal adenocarcinoma

PTH	parathyroid hormone
ROC	receiver operating characteristic
ROMA	risk of ovarian malignancy algorithm
SAA	serum amyloid A
sICAM	soluble intercellular adhesion molecule
sVCAM	soluble vascular cell adhesion molecule
TIMP	tissue inhibitor of metalloproteinase
TPA	tissue polypeptide antigen
tPAI-1	tissue plasminogen activator inhibitor 1
TPS	tissue polypeptide-specific antigen
TSH	thyroid stimulating hormone
UCr	urine creatinine
UGP	urinary gonadotropic peptide
VEGF	vascular endothelial growth factor

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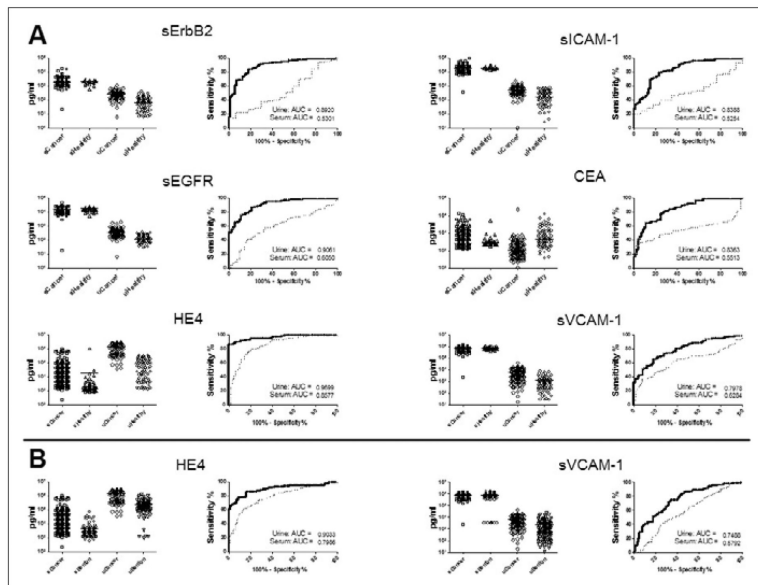
Fig. 1.
Comparative performance of urine and serum biomarkers in ovarian cancer. Urines obtained from 118 patients diagnosed with ovarian cancer, 118 women with benign pelvic conditions, and 72 healthy controls were evaluated using Luminex multiplexed immunoassays for 15 biomarkers found to be informative in 2 previous analyses in serum. Biomarker level distributions in urine and serum are presented along with ROC curves and AUC values (solid line: urine; dashed line: serum). A. Urine biomarker levels from ovarian cancer patients and healthy controls are compared with serum biomarker levels from a previous report (22). B. Urine biomarker levels from ovarian cancer patients and benign controls are compared with serum biomarker levels from a previous report (21). Biomarkers demonstrating significantly elevated AUC levels, based on 95% confidence intervals, in urine versus serum are shown.
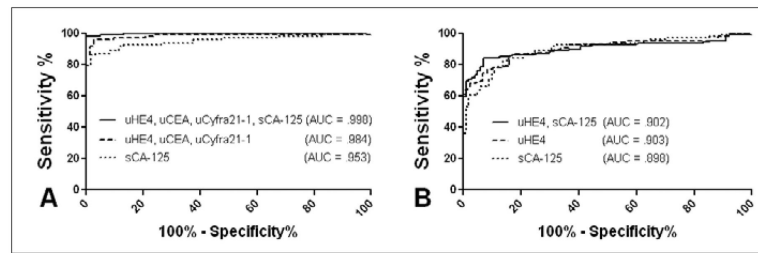
Fig. 2.
Performance of multimarker panels for the discrimination of ovarian cancer patients from controls. The top performing urine biomarker panels for the discrimination of ovarian cancer patients from healthy (A) and benign (B) controls were identified using the Metropolis-Monte Carlo (MMC) algorithm. Panel performance was compared to serum CA 125 measured in the same group. The impact of adding serum CA 125 to the top urine biomarker panel was evaluated for each comparison. s, serum; u, urine.
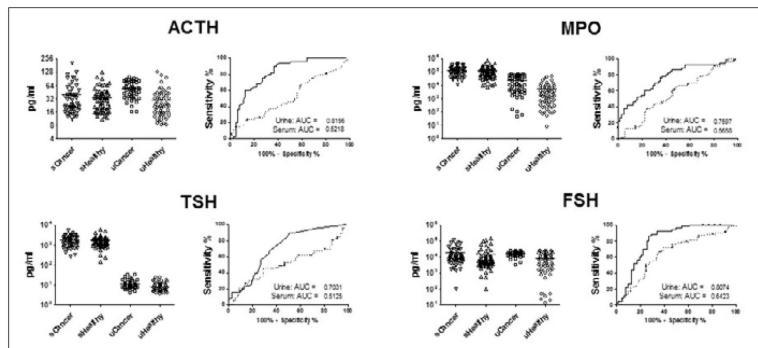
Fig. 3.

Comparative performance of urine and serum biomarkers in breast cancer. Urines obtained from 74 postmenopausal women diagnosed with stage I breast cancer and 90 age-matched healthy controls were evaluated using Luminex multiplexed immunoassays for 98 biomarkers previously tested in serum. Urine biomarker results were compared with serum biomarker results from a previous analysis (52). Biomarker level distributions in urine and serum are presented along with ROC curves and AUC values (solid line: urine; dashed line: serum). Biomarkers demonstrating significantly elevated AUC levels, based on 95% confidence intervals, in urine versus serum are shown. s, serum; u, urine.
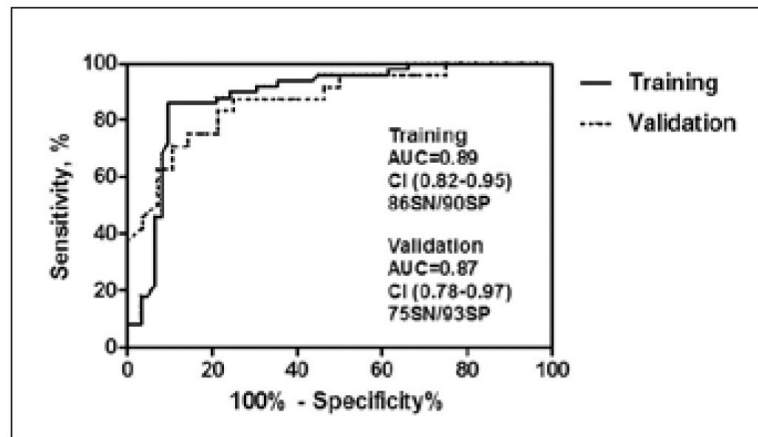
Fig. 4.
Performance of a urine multimarker panel in the discrimination of stage I breast cancer
patients from controls. The top performing urine biomarker panel for the discrimination of
stage I breast cancer patients from healthy controls was identified using the Metropolis-
Monte Carlo (MMC) algorithm. The performance of this 3-biomarker panel consisting of
FSH, MPO, and OC in distinct training and validation sets is presented according to ROC
analysis. AUC, area under the curve; CI, confidence interval; SN, sensitivity; SP, specificity.
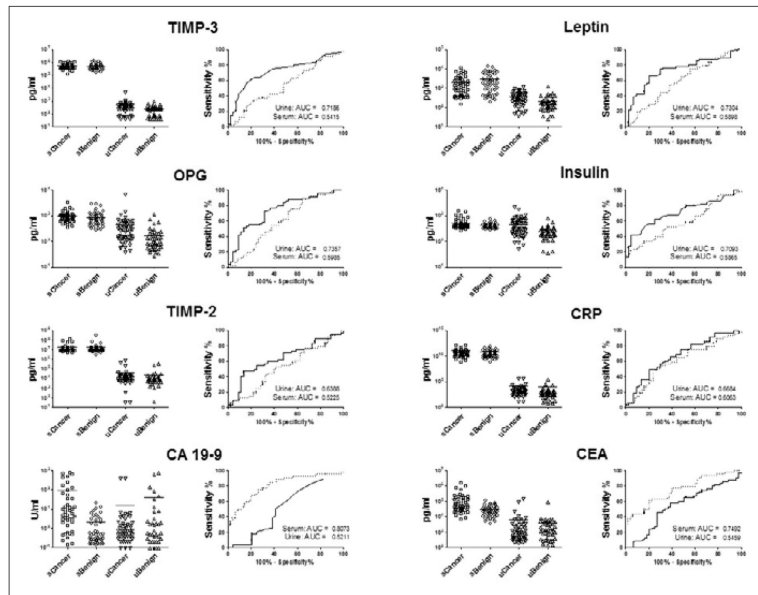
Fig. 5.
Comparative performance of urine and serum biomarkers in pancreatic cancer. Paired sera
and urines obtained from 55 patients diagnosed with pancreatic ductal adenocarcinoma and
44 patients diagnosed with a spectrum of benign pancreatic conditions were evaluated using
Luminex multiplexed immunoassays for 20 biomarkers. Biomarker level distributions in
urine and serum are presented along with ROC curves and AUC values (solid line: urine;
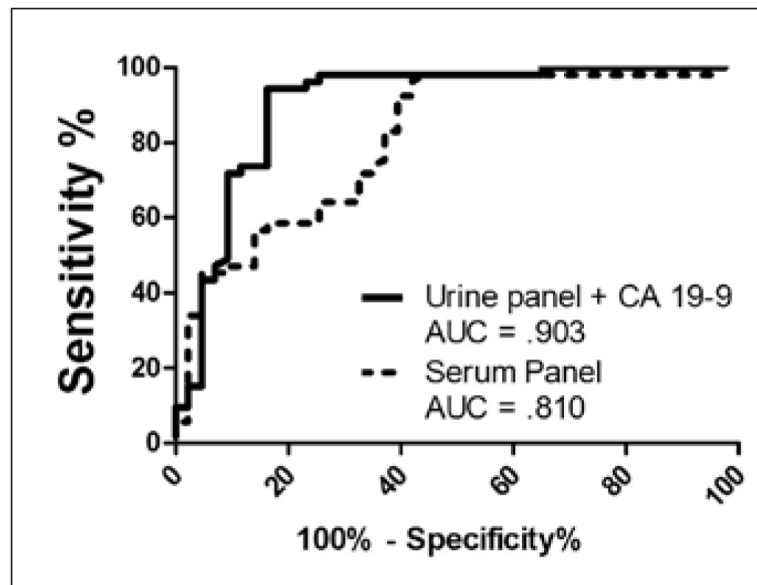dashed line: serum).

Fig. 6.
Performance of multimarker panels for the discrimination of pancreatic cancer patients from benign controls. The top performing urine and serum biomarker panels for the discrimination of pancreatic cancer patients from benign controls were identified using the Metropolis-Monte Carlo (MMC) algorithm. The best urine panel consisted of CA 125, insulin, and H-FABP while the top serum biomarker panel consisted of sICAM-1, TIMP-1, TIMP-4, CA 125, and CA 19-9. The addition of serum CA 19-9 to the urine panel resulted in optimal performance.

TABLE 1

PATIENT CHARACTERISTICS FOR URINE BIOMARKER ANALYSES

Group	Age (years)	Gender	Histology	Stage
Ovarian				
Healthy	Range: 49–85	Female (n=72)		
N=72	Median: 62			
Benign	Range: 47–86	Female (n=118)	Non-malignant neoplasms (n=41)	
N=118	Median: 62		Benign cysts (n=36)	
			LMP tumors (n=15)	
			Other benign lesions (n=26)	
Cancer	Range: 48–87	Female (n=108)	Serous (n=72)	I (n=5)
N=108	Median: 63		Endometrioid (n=7)	II (n=5)
			Mucinous (n=3)	III (n=85)
			Mixed (n=19)	IV (n=13)
			Undifferentiated/Unknown (n=7)	
Breast				
Healthy	Range: 49–84	Female (n=90)		
N=90[*]	Median: 67			
Cancer	Range: 49–92	Female (n=74)	Ductal (n=63)	I–II (n=74)
N=74[*]	Median: 63		Lobular (n=11)	
Pancreatic				
Healthy	Range: 22–65	Male (n=16)		
N=45	Median: 46	Female (n=29)		
Benign	Range: 32–85	Male (n=24)	Benign cyst (n=4)	
N=44	Median: 60	Female (n=20)	Pancreatitis (n=10)	
			Other (n=30)	
Cancer	Range: 42–91	Male (n=36)	PDAC (n=55)	I (n=2)
N=55	Median: 66	Female (n=19)		II (n=18)
				III (n=13)
				IV (n=22)

[*] Includes training and validation sets; LMP, low malignant potential; PDAC, pancreatic ductal adenocarcinoma