Supplementary Table 2. Shortlist of the 47 studies that have been retrieved from the literature, as being relevant to the research conducted the last 10 years regarding urinary proteomics biomarkers for Pca

Selection Criteria:

Relevant to the disease

Assessment of protein excretion levels in urine or levels in urinary exoxomes

Proteomics Analysis using Mass spectrometry and/ or Immuno-based assays

Bijnsdorp, I. V., et al. (2013). "Exosomal ITGA3 interferes with non-cancerous prostate cell functions and is increased in urine exosomes of metastatic prostate cancer patients." Journal of extracellular vesicles 2.

BACKGROUND: Cancer cells are able to change the protein expression and behavior of non-cancerous surrounding cells. Exosomes, secreted by prostate cancer (PCa) cells, may have a functional role in cancer metastasis and present a promising source for protein biomarkers. The aim of the present study was to identify which proteins in exosomes can influence non-cancerous cells, and to determine whether we can use urine exosomal proteins to identify high-risk PCa patients. METHOD: Exosomes were isolated by ultracentrifugation. Migration and invasion were studied by the transwell (invasion) assay. Proteomics was performed by LC-MS/MS and identified proteins were validated by Western blotting. Cellular uptake of fluorescent labeled PKH67-exosomes was measured by FACS. RESULTS: Based on comparative protein profiling by mass spectrometry-based proteomics of LNCaP- and PC3-exosomes, we selected ITGA3 and ITGB1, involved in migration/invasion, for further analyses. Inhibition of exosomal ITGA3 reduced the migration and invasion of non-cancerous prostate epithelial cells (prEC) almost completely. Cellular uptake of exosomes by prEC was higher with PC3-exosomes compared to LNCaP exosomes. Finally, ITGA3 and ITGB1 were more abundant in urine exosomes of metastatic patients ($p<0.05$), compared to benign prostate hyperplasia or PCa. CONCLUSION: These data indicate exosomal ITGA3 and ITGB1 may play a role in manipulating non-cancerous surrounding cells and that measurement of ITGA3 and ITGB1 in urine exosomes has the potential to identify patients with metastatic PCa in a non-invasive manner.

Bilgin Dogru, E., et al. (2014). "EMMPRIN and ADAM12 in prostate cancer: preliminary results of a prospective study." Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine 35(11): 11647-11653.

Extracellular metalloproteinase inducer (EMMPRIN) and a disintegrin and metalloproteinase (ADAM12) play a major role in cancer invasion and metastasis owing to the fact that they are directly related to the cell microenvironment and extracellular matrix (ECM) degradation. The aim of this study was to search for an answer to the question "whether the determination of EMMPRIN and ADAM12 values especially in urine may be helpful for the early diagnosis of prostate cancer without employing invasive methods" and also to check whether they may be useful for the determination of the patients with high metastasis risk. Peripheral blood and urine from 66 prostate cancer patients (40 local, 20 locally advanced, 6 metastatic) and 14 healthy controls were evaluated by enzyme-linked immunosorbent assay (ELISA) method. Serum EMMPRIN and ADAM12 values of the patients were seen to be statistically higher than the serum EMMPRIN and ADAM12 values of the healthy controls ($p=0.01$ and $p=0.001$, respectively). The urine ADAM12 levels were significantly higher in patients ($p=0.013$). No significant relationships were found between urine EMMPRIN values of the patients and the healthy controls ($p>0.05$). Positive correlation between urine EMMPRIN-urine ADAM12 tests was found in total patients group ($r=0.683$, $p=0.001$). Our preliminary results revealed that serum EMMPRIN and ADAM12 values and urine ADAM12 values may be useful markers in prostate cancer therapy. Due to the high correlation between these two tests, we are of the opinion that the use of urine ADAM12 in clinic may be sufficient and favorable together with prostate-specific antigen (PSA) for treatment.

Cao, D.-L., et al. (2011). "A Multiplex Model of Combining Gene-Based, Protein-Based, and Metabolite-Based With Positive and Negative Markers in Urine for the Early Diagnosis of Prostate Cancer." Prostate 71(7): 700-710.

BACKGROUND. Multiplex urine-based assay emerged outperforms single biomarker (e. g., prostate-specific antigen, PSA) for predicting prostate cancer (CaP), whereas its combined mode has to be fully optimized. Our aim is to determine whether a strategy of combining gene-based, protein-based, metabolite-based with positive, negative makers in urine could optimize a multiplex model for detecting CaP. METHODS. Using quantitative PCR, Western blot, and liquid chromatography-mass spectrometry, expression patterns of PCA3, TMPRSS2: ERG, Annexin A3, Sarcosine, and urine PSA were evaluated in urine samples from 86 untreated patients with CaP and 45 patients with no evidence of malignancy. Multivariate logistic regression analysis was used to generate a final model and receiver-operating characteristic (ROC) analysis and special bootstrap software to assess diagnostic performance of tested variables. RESULTS. The expression patterns of PCA3, TMPRSS2: ERG, Annexin A3, Sarcosine, and a panel including these biomarkers were significant predictors of CaP both in patients with PSA 4-10 ng/ml and in all patients (all P < 0.05). Employing ROC analysis, the area under the curves of the panel in these both cohorts were 0.840 and 0.856, respectively, which outperform that of any single biomarker (PCA3: 0.733 and 0.739; TMPRSS2: ERG: 0.720 and 0.732; Annexin A3: 0.716 and 0.728; Sarcosine: 0.659 and 0.665, respectively). CONCLUSIONS. Compared with single biomarker, the multiplex model including PCA3, TMPRSS2: ERG, Annexin A3 and Sarcosine adds even more to the diagnostic performance for predicting CaP. Further validation experiments and optimization for the strategy of constructing this model are warranted. Prostate 71: 700-710, 2011. (C) 2010 Wiley-Liss, Inc.

Casanova-Salas, I., et al. (2015). "MiR-187 Targets the Androgen-Regulated Gene ALDH1A3 in Prostate Cancer." PloS one 10(5): e0125576-e0125576.

miRNAs are predicted to control the activity of approximately 60% of all protein-coding genes participating in the regulation of several cellular processes and diseases, including cancer. Recently, we have demonstrated that miR-187 is significantly downregulated in prostate cancer (PCa) and here we propose a proteomic approach to identify its potential targets. For this purpose, PC-3 cells were transiently transfected with miR-187 precursor and miRNA mimic negative control. Proteins were analyzed by a two-dimensional difference gel electrophoresis (2D-DIGE) and defined as differentially regulated if the observed fold change was ±1.06. Then, MALDI-TOF MS analysis was performed after protein digestion and low abundance proteins were identified by LC-MS/MS. Peptides were identified by searching against the Expasy SWISS PROT database, and target validation was performed both in vitro by western blot and qRT-PCR and in clinical samples by qRT-PCR, immunohistochemistry and ELISA. DIGE analysis showed 9 differentially expressed spots (p<0.05) and 7 showed a down-regulated expression upon miR-187 re-introduction. Among these targets we identified aldehyde dehydrogenase 1A3 (ALDH1A3). ALDH1A3 expression was significantly downregulated in PC3, LNCaP and DU-145 cells after miR-187 re-introduction. Supporting these data, the expression of ALDH1A3 was found significantly (p<0.0001) up-regulated in PCa samples and inversely correlated (p<0.0001) with miR-187 expression, its expression being directly associated with Gleason score (p = 0.05). The expression of ALDH1A3 was measured in urine samples to evaluate the predictive capability of this biomarker for the presence of PCa and, at a signification level of 10%, PSA and also ALDH1A3 were significantly associated with a positive biopsy of PCa. In conclusion, our data illustrate for the first time the role of ALDH1A3 as a miR-187 target in PCa and provide insights in the utility of using this protein as a new biomarker for PCa.

Chen, J., et al. (2013). "Identification, prioritization, and evaluation of glycoproteins for aggressive prostate cancer using quantitative glycoproteomics and antibody-based assays on tissue specimens." Proteomics 13(15): 2268-2277.

Prostate cancer is highly heterogeneous in nature; while the majority of cases are clinically insignificant, some cases are lethal. Currently, there are no reliable screening methods for aggressive prostate cancer. Since most established serum and urine biomarkers are glycoproteins secreted or leaked from the diseased tissue, the current study seeks to identify glycoprotein markers specific to aggressive prostate cancer using tissue specimens. With LC-MS/MS glycoproteomic analysis, we identified 350 glycopeptides with 17 being altered in aggressive prostate cancer. ELISA assays were developed/purchased to evaluate four candidates, that is, cartilage oligomeric

matrix protein (COMP), periostin, membrane primary amine oxidase (VAP-1), and cathepsin L, in independent tissue sets. In agreement with the proteomic analysis, we found that COMP and periostin expressions were significantly increased in aggressive prostate tumors while VAP-1 expression was significantly decreased in aggressive tumor. In addition, the expression of these proteins in prostate metastases also follows the same pattern observed in the proteomic analysis. COMP and periostin and decrease in VAP-1 expression in the prostate may be associated with aggressive prostate cancer.

Chen, M., et al. (2011). "The Discovery of Putative Urine Markers for the Specific Detection of Prostate Tumor by Integrative Mining of Public Genomic Profiles." PloS one 6(12).

Urine has emerged as an attractive biofluid for the noninvasive detection of prostate cancer (PCa). There is a strong imperative to discover candidate urinary markers for the clinical diagnosis and prognosis of PCa. The rising flood of various omics profiles presents immense opportunities for the identification of prospective biomarkers. Here we present a simple and efficient strategy to derive candidate urine markers for prostate tumor by mining cancer genomic profiles from public databases. Prostate, bladder and kidney are three major tissues from which cellular matters could be released into urine. To identify urinary markers specific for PCa, upregulated entities that might be shed in exosomes of bladder cancer and kidney cancer are first excluded. Through the ontology-based filtering and further assessment, a reduced list of 19 entities encoding urinary proteins was derived as putative PCa markers. Among them, we have found 10 entities closely associated with the process of tumor cell growth and development by pathway enrichment analysis. Further, using the 10 entities as seeds, we have constructed a protein-protein interaction (PPI) subnetwork and suggested a few urine markers as preferred prognostic markers to monitor the invasion and progression of PCa. Our approach is amenable to discover and prioritize potential markers present in a variety of body fluids for a spectrum of human diseases.

Christensen, E., et al. (2009). "Longitudinal Cytokine Expression during IMRT for Prostate Cancer and Acute Treatment Toxicity." Clinical Cancer Research 15(17): 5576-5583.

Purpose: Proteomic profiling of patients undergoing intensity-modulated radiotherapy (IMRT) for prostate cancer can identify unique biomarkers that reflect acute toxicity in normal tissues. Our objectives were to measure inflammatory cytokine proteins during IMRT and assess the variability of individual proteomic signatures. Experimental Design: Forty-two patients with intermediate-risk prostate cancer were recruited as follows: group 1, definitive IMRT (78 Gy in 39 fractions, n = 22), and group 2, IMRT postprostatectomy (66 Gy in 33 fractions, n = 20). Blood/urine samples were collected at baseline and weekly during IMRT. Acute toxicity was graded weekly during radiotherapy using CTC-AE v3.0 criteria. Multiplexed immunoassays were used to quantify cytokines including granulocyte macrophage colony-stimulating factor, IFN-gamma, tumor necrosis factor-alpha, interleukin (IL)-1 alpha, IL-2, IL6, IL-8, IL-10, and IL-12p70. Results: We observed positive correlations between cytokine expression between serum and plasma, but not between serum/plasma and urine. The Mann-Whitney test showed a significant increase in IFN-gamma and IL-6 during IMRT (P = 0.0077, 0.0035). Increasing IL-2 and IL-1 expression were associated with increased probability of acute gastrointestinal and genitourinary toxicity, respectively. Conclusions: Determination of radiation-response signatures is feasible using multiplexed immunoassays and is a promising predictive early biomarker of toxicity outcomes. (Clin Cancer Res 2009;15(17):5576-83)

Christiansen, H., et al. (2007). "Increase of hepcidin plasma and urine levels is associated with acute proctitis and changes in hemoglobin levels in primary radiotherapy for prostate cancer." Journal of Cancer Research and Clinical Oncology 133(5): 297-304.

Purpose To analyse hepcidin serum and urine levels during radiotherapy for prostate cancer. Methods In 18 patients undergoing radiotherapy for prostate cancer, blood, plasma, and urine samples were taken before and during radiotherapy. Complete blood cell count, pro-hepcidin-, ferritin-, transferrin-, IL-1 beta-, IL-6-, and TNF-alpha concentration was determined. Pro-hepcidin concentration was additionally measured in urine samples. Toxicity was evaluated weekly. Differences among tested factors were tested by Wilcoxon rank sign test for paired data. Results In ten patients developing acute radiation-induced proctitis, a significant increase in pro-hepcidin, IL-6, and TNF-alpha plasma levels ($p < 0.05$) was detected. Pro-hepcidin urine levels also showed a strong trend towards increase ($p = 0.06$). Concurrently, hemoglobin, and leucocytes were significantly decreased in the patients with acute proctitis ($p < 0.05$). In eight patients showing no symptoms of proctitis, solely a significant decrease for leucocytes was detected. Additive, these patients showed a significant increase of ferritin, and a decrease of transferrin levels ($p < 0.05$). Conclusions Hepcidin levels

are increased and hemoglobin is decreased during radiotherapy for prostate cancer in patients who develop acute proctitis. Radiation-induced expression of cytokines may be responsible for increased hepcidin expression in the liver. Regulation of iron metabolism by hepcidin may be an underestimated response in radiotherapy.

Davalieva, K., et al. (2015). "Proteomics analysis of urine reveals acute phase response proteins as candidate diagnostic biomarkers for prostate cancer." Proteome Science 13.

Despite the overall success of prostate specific antigen (PSA) in screening and detection of prostate cancer (PCa), its use has been limited due to the lack of specificity. The principal driving goal currently within PCa research is to identify non-invasive biomarker(s) for early detection of aggressive tumors with greater sensitivity and specificity than PSA. In this study, we focused on identification of non-invasive biomarkers in urine with higher specificity than PSA. We tested urine samples from PCa and benign prostatic hyperplasia (BPH) patients by 2-D DIGE coupled with MS and bioinformatics analysis. Statistically significant ($p < 0.05$), 1.8 fold variation or more in abundance, showed 41 spots, corresponding to 23 proteins. The Ingenuity Pathway Analysis showed significant association with the Acute Phase Response Signaling pathway. Nine proteins with differential abundances were included in this pathway: AMBP, APOA1, FGA, FGG, HP, ITIH4, SERPINA1, TF and TTR. The expression pattern of 4 acute phase response proteins differed from the defined expression in the canonical pathway. The urine levels of TF, AMPB and HP were measured by immunoturbidimetry in an independent validation set. The concentration of AMPB in urine was significantly higher in PCa while levels of TF and HP were opposite ($p < 0.05$). The AUC for the individual proteins ranged from 0.723 to 0.754. The combination of HP and AMBP yielded the highest accuracy (AUC = 0.848), greater than PSA. The proposed biomarker set is quickly quantifiable and economical with potential to improve the sensitivity and specificity of PCa detection.

Dudderidge, T. J., et al. (2010). "Diagnosis of prostate cancer by detection of minichromosome maintenance 5 protein in urine sediments." British Journal of Cancer 103(5): 701-707.

BACKGROUND: The accuracy of prostate-specific antigen (PSA) testing in prostate cancer detection is constrained by low sensitivity and specificity. Dysregulated expression of minichromosome maintenance (Mcm) 2-7 proteins is an early event in epithelial multistep carcinogenesis and thus MCM proteins represent powerful cancer diagnostic markers. In this study we investigate Mcm5 as a urinary biomarker for prostate cancer detection. METHODS: Urine was obtained from 88 men with prostate cancer and from two control groups negative for malignancy. A strictly normal cohort included 28 men with complete, normal investigations, no urinary calculi and serum PSA <2 ngml(-1). An expanded control cohort comprised 331 men with a benign final diagnosis, regardless of PSA level. Urine was collected before and after prostate massage in the cancer patient cohort. An immunofluorometric assay was used to measure Mcm5 levels in urine sediments. RESULTS: The Mcm5 test detected prostate cancer with 82% sensitivity (confidence interval (CI) 72-89%) and with a specificity ranging from 73 (CI=68-78%) to 93% (CI=76-99%). Prostate massage led to increased Mcm5 signals compared with pre-massage samples (median 3440 (interquartile range (IQR) 2280 to 5220) vs 2360 (IQR <1800 to 4360); P=0.009), and was associated with significantly increased diagnostic sensitivity (82 vs 60%; P=0.012). CONCLUSIONS: Urinary Mcm5 detection seems to be a simple, accurate and noninvasive method for identifying patients with prostate cancer. Large-scale prospective trials are now required to evaluate this test in diagnosis and screening. British Journal of Cancer (2010) 103, 701-707. doi:10.1038/sj.bjc.6605785 www.bjcancer.com Published online 20 July 2010 (C) 2010 Cancer Research UK

Fujita, K., et al. (2009). "Endoglin (CD105) as a urinary and serum marker of prostate cancer." International Journal of Cancer 124(3): 664-669.

We have previously shown that endoglin (CD105) is upregulated in prostatic fluid of men with large volume prostate cancer. We chose to assess endoglin levels in urine and serum from men with prostate cancer or at increased risk for the disease: Urine samples were collected after digital rectal examination (DRE) from 99 men whose cancer status was confirmed by biopsy, and serum samples were collected from 20 men without prostate cancer at low risk for the disease and from 69 men diagnosed with prostate cancer that subsequently underwent radical prostatectomy (30 pT2, 39 pT3). Endoglin levels were assessed by ELISA. Urinary endoglin was elevated in men with biopsy-positive prostate cancer compared to biopsy-negative men ($p = 0.0014$). Urinary endoglin levels in men with prostate cancer correlated with radical prostatectomy tumor volume. The area under the receiver operating characteristic (ROC) curve was 0.72 for urinary endoglin and 0.50 for serum prostate-specific antigen (PSA; sensitivity for cancer detection 73%, specificity 63%). There were no differences in serum endoglin between normal and cancer cases, but there were increases in serum endoglin in non-organ confined (NOC, pT3+) versus organ-confined (OC, pT2) cases ($p = 0.0004$). The area under the ROC curve was 0.75 for serum endoglin and

0.63 for PSA for predicting NOC status, with a sensitivity of 67% and a specificity of 80%. In conclusion, elevations in post-DRE urinary endoglin suggest there may be value in further studying endoglin as a urinary biomarker of prostate cancer. Endoglin levels in both urine and serum may aid in prostate cancer detection and prognostication. (C) 2008 Wiley-Liss. Inc.

Fujita, K., et al. (2011). "Immunomodulatory IL-18 binding protein is produced by prostate cancer cells and its levels in urine and serum correlate with tumor status." International Journal of Cancer 129(2): 424-432.

Cytokines may play a role in the initiation and progression of prostate cancer. A cytokine antibody array was previously applied to prostatic fluid obtained from patients with prostate cancer, and interleukin 18 binding protein (IL-18BP), a potent inhibitor of interleukin 18, was noted to be significantly upregulated in cases with large volume disease. We sought to further characterize the association of IL-18BP with prostate cancer and determine whether IL-18BP levels in patient serum and urine samples had clinical relevance. IL-18BP was expressed and secreted by the prostate cancer cell lines DU145 and PC3 but not by LNCaP and CWR22, upon interferon-gamma (IFN-gamma) stimulation. IFN-gamma-induced secretion of IL-18BP was enhanced by added TNF-alpha, IFN-alpha and IFN-beta. The IL-18BP secreted from DU145 and PC3 functionally inhibited IL-18. Immunohistochemical analyses showed positive IL-18BP staining in prostate cancer cells as well as in macrophages in radical prostatectomy specimens. Significant differences in urinary IL-18BP levels (normalized by total protein) collected post-DRE were found between cases with and without cancer on biopsy (p = 0.02) and serum IL-18BP levels correlated with Gleason score (p = 0.03). Our finding of elevated IL-18BP secretion from prostate cancer cells suggests an attempt by cancer to escape immune surveillance. IL-18BP merits further study as a marker of aggressive prostate cancer and as a therapeutic target.

Geisler, C., et al. (2015). "Identification and validation of potential new biomarkers for prostate cancer diagnosis and prognosis using 2D-DIGE and MS." BioMed research international 2015: 454256-454256.

This study was designed to identify and validate potential new biomarkers for prostate cancer and to distinguish patients with and without biochemical relapse. Prostate tissue samples analyzed by 2D-DIGE (two-dimensional difference in gel electrophoresis) and mass spectrometry (MS) revealed downregulation of secernin-1 (P<0.044) in prostate cancer, while vinculin showed significant upregulation (P<0.001). Secernin-1 overexpression in prostate tissue was validated using Western blot and immunohistochemistry while vinculin expression was validated using immunohistochemistry. These findings indicate that secernin-1 and vinculin are potential new tissue biomarkers for prostate cancer diagnosis and prognosis, respectively. For validation, protein levels in urine were also examined by Western blot analysis. Urinary vinculin levels in prostate cancer patients were significantly higher than in urine from nontumor patients (P=0.006). Using multiple reaction monitoring-MS (MRM-MS) analysis, prostatic acid phosphatase (PAP) showed significant higher levels in the urine of prostate cancer patients compared to controls (P=0.012), while galectin-3 showed significant lower levels in the urine of prostate cancer patients with biochemical relapse, compared to those without relapse (P=0.017). Three proteins were successfully differentiated between patients with and without prostate cancer and patients with and without relapse by using MRM. Thus, this technique shows promise for implementation as a noninvasive clinical diagnostic technique.

Haj-Ahmad, T. A., et al. (2014). "Potential Urinary Protein Biomarker Candidates for the Accurate Detection of Prostate Cancer among Benign Prostatic Hyperplasia Patients." Journal of Cancer 5(2): 103-114.

Globally, Prostate cancer (PCa) is the most frequently occurring non-cutaneous cancer, and is the second highest cause of cancer mortality in men. Serum prostate specific antigen (PSA) has been the standard in PCa screening since its approval by the American Food & Drug Administration (FDA) in 1994. Currently, PSA is used as an indicator for PCa - patients with a serum PSA level above 4ng/mL will often undergo prostate biopsy to confirm cancer. Unfortunately fewer than similar to 30% of these men will biopsy positive for cancer, meaning that the majority of men undergo invasive biopsy with little benefit. Despite PSA's notoriously poor specificity (33%), there is still a significant lack of credible alternatives. Therefore an ideal biomarker that can specifically detect PCa at an early stage is urgently required. The aim of this study was to investigate the potential of using deregulation of urinary proteins in order to detect Prostate Cancer (PCa) among Benign Prostatic Hyperplasia (BPH). To identify the protein signatures specific for PCa, protein expression profiling of 8 PCa patients, 12 BPH patients and 10 healthy males was carried out using LC-MS/MS. This was followed by validating relative expression levels of proteins present in urine among all the patients using quantitative real time-PCR. This was followed by validating

relative expression levels of proteins present in urine among all the patients using quantitative real time-PCR. This approach revealed that significant the down-regulation of Fibronectin and TP53INP2 was a characteristic event among PCa patients. Fibronectin mRNA down-regulation, was identified as offering improved specificity (50%) over PSA, albeit with a slightly lower although still acceptable sensitivity (75%) for detecting PCa. As for TP53INP2 on the other hand, its down-regulation was moderately sensitive (75%), identifying many patients with PCa, but was entirely non-specific (7%), designating many of the benign samples as malignant and being unable to accurately identify more than one negative.

Hamelin-Peyron, C., et al. (2014). "Prostate cancer biomarker annexin A3 detected in urines obtained following digital rectal examination presents antigenic variability." Clinical Biochemistry 47(10-11): 901-908.

Objectives: Annexin A3 (ANXA3) is a potential marker for prostate cancer (PCa). We aimed to develop robust immunoassays suitable for quantifying ANXA3 in urine samples obtained following digital rectal examination (DRE) in order to facilitate the diagnostic performance evaluation of this marker. Design and methods: Anti-ANXA3 monoclonal antibodies were generated and their epitopes mapped. Two different ANXA3 assay prototypes were established on the VIDAS (R) automated immunoanalyser and analytical validation was carried out using post-DRE urine samples obtained from patients with PCa (n = 23) or benign prostate hyperplasia (n = 31). Results: The assays had the same capture antibody (TGC44) but different detection antibodies (13A12 or 5C5), recognizing novel distinct epitopes. Both had a lower limit of quantification <1 ng/mL and were highly specific for ANXA3, not cross-reacting with other annexins. Interassay imprecision was <= 11% and <= 15% for 13A12 and 5C5 assays, respectively. Surprisingly, a total lack of correlation was observed between ANXA3 levels measured by these two assays in post-DRE urines, indicating detection of distinct antigenic variants. Two freeze-thaw cycles did not affect analyte stability in either assay, whereas a lack of stability of antigenic variants was observed when samples were stored at -80 degrees C for 1 month. Conclusions: Two different antigenic variants of ANXA3 are present in post-DRE urines and their clinical significance for diagnosis of prostate cancer should be further investigated. These variants are not stable over time in samples preserved at -80 degrees C. Until this issue is resolved, ANXA3 should only be measured in freshly collected samples.

He, J., et al. (2015). "Analytical platform evaluation for quantification of ERG in prostate cancer using protein and mRNA detection methods." Journal of Translational Medicine 13.

Background: The established methods for detecting prostate cancer (CaP) are based on tests using PSA (blood), PCA3 (urine), and AMACR (tissue) as biomarkers in patient samples. The demonstration of ERG oncoprotein overexpression due to gene fusion in CaP has thus provided ERG as an additional biomarker. Based on this, we hypothesized that ERG protein quantification methods can be of use in the diagnosis of prostate cancer. Methods: An antibody-free assay for ERG3 protein detection was developed based on PRISM (high-pressure high-resolution separations with intelligent selection and multiplexing)-SRM (selected reaction monitoring) mass spectrometry. We utilized TMPRSS2-ERG positive VCaP and TMPRSS2-ERG negative LNCaP cells to simulate three different sample types (cells, tissue, and post-DRE urine sediment). Enzyme-linked immunosorbent assay (ELISA), western blot, NanoString, and qRT-PCR were also used in the analysis of these samples. Results: Recombinant ERG3 protein spiked into LNCaP cell lysates could be detected at levels as low as 20 pg by PRISM-SRM analysis. The sensitivity of the PRISM-SRM assay was approximately 10,000 VCaP cells in a mixed cell population model of VCaP and LNCaP cells. Interestingly, ERG protein could be detected in as few as 600 VCaP cells spiked into female urine. The sensitivity of the in-house ELISA was similar to the PRISM-SRM assay, with detection of 30 pg of purified recombinant ERG3 protein and 10,000 VCaP cells. On the other hand, qRT-PCR exhibited a higher sensitivity, as TMPRSS2-ERG transcripts were detected in as few as 100 VCaP cells, in comparison to NanoString methodologies which detected ERG from 10,000 cells. Conclusions: Based on this data, we propose that the detection of both ERG transcriptional products with RNA-based assays, as well as protein products of ERG using PRISM-SRM assays, may be of clinical value in developing diagnostic and prognostic assays for prostate cancer given their sensitivity, specificity, and reproducibility.

Jayapalan, J. J., et al. (2013). "Urine of patients with early prostate cancer contains lower levels of light chain fragments of inter-alpha-trypsin inhibitor and saposin B but increased expression of an inter-alpha-trypsin inhibitor heavy chain 4 fragment." Electrophoresis 34(11): 1663-1669.

The present study was aimed at the identification of proteins that are differentially expressed in the urine of patients with prostate cancer (PCa), those with benign prostatic hyperplasia (BPH) and age-matched healthy male control subjects. Using a combination of 2DE and MS/MS, significantly lower expression of urinary saposin B and two different fragments of inter-alpha-trypsin inhibitor light chain (ITIL) was demonstrated in the PCa patients compared to the controls. However, only one of the ITIL fragments was significantly different between the PCa and BPH patients. When image analysis was performed on urinary proteins that were transferred onto NC membranes and detected using a lectin that binds to O-glycans, a truncated fragment of inter-alpha-trypsin inhibitor heavy chain4 was the sole protein found to be significantly enhanced in the PCa patients compared to the controls. Together, these urinary peptide fragments might be useful complementary biomarkers to indicate PCa as well as to distinguish it from BPH, although further epidemiological evidence on the specificity and sensitivity of the protein candidates is required.

Jedinak, A., et al. (2015). "Novel non-invasive biomarkers that distinguish between benign prostate hyperplasia and prostate cancer." Bmc Cancer 15.

Background: The objective of this study was to discover and to validate novel noninvasive biomarkers that distinguish between benign prostate hyperplasia (BPH) and localized prostate cancer (PCa), thereby helping to solve the diagnostic dilemma confronting clinicians who treat these patients. Methods: Quantitative iTRAQ LC/LC/MS/MS analysis was used to identify proteins that are differentially expressed in the urine of men with BPH compared with those who have localized PCa. These proteins were validated in 173 urine samples from patients diagnosed with BPH (N = 83) and PCa (N = 90). Multivariate logistic regression analysis was used to identify the predictive biomarkers. Results: Three proteins, beta 2M, PGA3, and MUC3 were identified by iTRAQ and validated by immunoblot analyses. Univariate analysis demonstrated significant elevations in urinary beta 2M (P < 0.001), PGA3 (P = 0.006), and MUC3 (P = 0.018) levels found in the urine of PCa patients. Multivariate logistic regression analysis revealed AUC values ranging from 0.618 for MUC3 (P = 0.009), 0.625 for PGA3 (P < 0.008), and 0.668 for beta 2M (P < 0.001). The combination of all three demonstrated an AUC of 0.710 (95% CI: 0.631 - 0.788, P < 0.001); diagnostic accuracy improved even more when these data were combined with PSA categories (AUC = 0.812, (95% CI: 0.740 - 0.885, P < 0.001). Conclusions: Urinary beta 2M, PGA3, and MUC3, when analyzed alone or when multiplexed with clinically defined categories of PSA, may be clinically useful in noninvasively resolving the dilemma of effectively discriminating between BPH and localized PCa.

Katafigiotis, I., et al. (2012). "Zinc alpha 2-glycoprotein as a potential novel urine biomarker for the early diagnosis of prostate cancer." Bju International 110(11B): E688-E693.

OBJECTIVE To examine the potential utility as a novel biomarker in the urine of zinc a 2-glygoprotein (ZAG) for the early diagnosis of prostate cancer. PATIENTS AND METHODS The urine of 127 consecutive candidates for a transrectal ultrasound prostatic biopsy with a mean age of 65.7 +/- 8.7 years and mean PSA 9.1 +/- 5.3 ng/mL was collected. Western blot analysis and immunohistochemistry for ZAG were performed. Receiver operating characteristic curves and logistic regression models were used to estimate the predictive ability of ZAG and to determine the optimal sensitivity and specificity by using various cut-off values for the prediction of prostate cancer. RESULTS In all, 42 patients had prostate cancer, 29 showed high grade prostatic intraepithelial neoplasia and 56 were negative. Receiver operating characteristic curve analysis showed a significant predictive ability of ZAG for prostate cancer. The area under the curve (AUC) for the prediction of prostate cancer was 0.68 (95% CI 0.59-0.78). The combination of ZAG with PSA showed a significant improvement in the predictive ability (P = 0.010), with AUC equal to 0.75 (95% CI 0.66-0.85). Separate analysis in patients with PSA levels of 4-10 ng/mL (70.1%) showed that ZAG had a discriminative power with AUC equal to 0.68. The optimal cut-off was 1.13 for ZAG, which corresponded to 6.88 times greater odds for prostate cancer. CONCLUSIONS Urine detected ZAG showed promising results in the prediction of prostate cancer. Further validation is required to establish ZAG as a novel biomarker.

Killick, E., et al. (2013). "Role of Engrailed-2 (EN2) as a prostate cancer detection biomarker in genetically high risk men." Scientific Reports 3.

Controversy surrounds the use of PSA as a biomarker for prostate cancer detection, leaving an unmet need for a novel biomarker in this setting; urinary EN2 may identify individuals with clinically relevant prostate cancer. Male BRCA1 and BRCA2 mutation carriers are at increased risk of clinically significant prostate cancer and may benefit

from screening. Urine samples from 413 BRCA1 and BRCA2 mutation carriers and controls were evaluated. Subjects underwent annual PSA screening with diagnostic biopsy triggered by PSA >3.0 ng/ml; 21 men were diagnosed with prostate cancer. Urinary EN2 levels were measured by ELISA and had a sensitivity of 66.7% and specificity of 89.3% for cancer detection. There was no statistically significant difference in EN2 levels according to genetic status or Gleason score. Urinary EN2 may be useful as a non-invasive early biomarker for prostate cancer detection in genetically high-risk individuals.

Kim, Y., et al. (2012). "Identification of Differentially Expressed Proteins in Direct Expressed Prostatic Secretions of Men with Organ-confined Versus Extracapsular Prostate Cancer." Molecular & Cellular Proteomics 11(12): 1870-1884.

Current protocols for the screening of prostate cancer cannot accurately discriminate clinically indolent tumors from more aggressive ones. One reliable indicator of outcome has been the determination of organ-confined versus nonorgan-confined disease but even this determination is often only made following prostatectomy. This underscores the need to explore alternate avenues to enhance outcome prediction of prostate cancer patients. Fluids that are proximal to the prostate, such as expressed prostatic secretions (EPS), are attractive sources of potential prostate cancer biomarkers as these fluids likely bathe the tumor. Direct-EPS samples from 16 individuals with extracapsular (n = 8) or organ-confined (n = 8) prostate cancer were used as a discovery cohort, and were analyzed in duplicate by a nine-step MudPIT on a LTQ-Orbitrap XL mass spectrometer. A total of 624 unique proteins were identified by at least two unique peptides with a 0.2% false discovery rate. A semiquantitative spectral counting algorithm identified 133 significantly differentially expressed proteins in the discovery cohort. Integrative data mining prioritized 14 candidates, including two known prostate cancer biomarkers: prostate-specific antigen and prostatic acid phosphatase, which were significantly elevated in the direct-EPS from the organ-con-fined cancer group. These and five other candidates (SFN, MME, PARK7, TIMP1, and TGM4) were verified by Western blotting in an independent set of direct-EPS from patients with biochemically recurrent disease (n = 5) versus patients with no evidence of recurrence upon follow-up (n = 10). Lastly, we performed proof-of-concept SRM-MS-based relative quantification of the five candidates using unpurified heavy isotope-labeled synthetic peptides spiked into pools of EPS-urines from men with extracapsular and organ-confined prostate tumors. This study represents the first efforts to define the direct-EPS proteome from two major subclasses of prostate cancer using shotgun proteomics and verification in EPS-urine by SRM-MS. Molecular & Cellular Proteomics 11: 10.1074/mcp.M112.017889, 1870-1884, 2012.

Kiprijanovska, S., et al. (2014). "Mapping and Identification of the Urine Proteome of Prostate Cancer Patients by 2D PAGE/MS." International journal of proteomics 2014: 594761-594761.

Proteome analysis of the urine has shown that urine contains disease-specific information for a variety of urogenital system disorders, including prostate cancer (PCa). The aim of this study was to determine the protein components of urine from PCa patients. Urine from 8 patients with clinically and histologically confirmed PCa was analyzed by conventional 2D PAGE. The MS identification of the most prominent 125 spots from the urine map revealed 45 distinct proteins. According to Gene Ontology, the identified proteins are involved in a variety of biological processes, majority of them are secreted (71%), and half of them are enzymes or transporters. Comparison with the normal urine proteome revealed 11 proteins distinctive for PCa. Using Ingenuity Pathways Analysis, we have found 3 proteins (E3 ubiquitin-protein ligase rififylin, tumor protein D52, and thymidine phosphorylase) associated with cellular growth and proliferation (p = 8.35 * 10(-4) - 3.41 * 10(-2)). The top network of functional associations between 11 proteins was Cell Death and Survival, Cell-To-Cell Signaling and Interaction, and System Development and Function (p = 10(-30)). In summary, we have created an initial proteomic map of PCa patient's urine. The results from this study provide some leads to understand the molecular bases of prostate cancer.

Li, C., et al. (2015). "Quantitative urinary proteomics using stable isotope labelling by peptide dimethylation in patients with prostate cancer." Analytical and Bioanalytical Chemistry 407(12): 3393-3404.

Prostate cancer (PCa) is the most commonly diagnosed malignancy in men. The current prevalent diagnosis method, prostate-specific antigen (PSA) screening test, has low sensitivity, specificity and is poor at predicting the grade of disease. Thus, new biomarkers are urgently needed to improve the PCa diagnosis and staging for the management of patients. The aim of this study is to investigate the first voided urinary sample after massage for biomarker discovery for PCa. In this work, untargeted metabolomic profiling of the first voided urinary sample after massage from 28 confirmed prostate cancer patients, 20 benign enlarged prostate patients and 6 healthy

volunteers was performed using liquid chromatography coupled to high-resolution tandem mass spectrometry (LC-MS/MS). Single and multiple peptide protein and cross-linking molecules were identified using PEAKS software. Analytical and diagnostic performance was tested using the Student's t test, Benjamini Hochberg correction and the receiver operating characteristic (ROC) curves. Using differential display analysis to compare peptides and cross-linking molecules of urinary samples between patients with benign, enlarged prostate and malignant cancer, we identified multiple peptides derived from osteopontin (SPP1) and prothrombin (F2) that are lower in PCa patients than in benign and enlarged prostate. The diagnosis accuracies of SPP1 and F2 peptides are 0.65-0.77 and 0.68-0.72, respectively. In addition to this, there are significant differences between PCa and benign/enlarged prostate patients in pyridinoline (PYD) and deoxypyridinoline (DPD) (p value = 0.001). Differences also, as shown in the excretion of these molecules for different stages of PCa (p value = 0.04) as the level of DPD and DPD/PYD ratio, were high in patients with locally advanced tumours. The study underscores the importance of proteomics analysis, and our results demonstrate that a urinary-based in depth proteomic approach allows the potential identification of dysregulated pathways and diagnostic biomarkers.

Lu, Q., et al. (2009). "Identification of Extracellular delta-Catenin Accumulation for Prostate Cancer Detection." Prostate 69(4): 411-418.

BACKGROUND. Prostate cancer is the second leading cause of cancer death in men, and early detection is essential to reduce mortality and increase survival. delta-Catenin is a unique beta-catenin superfamily protein primarily expressed in the brain but is upregulated in human prostatic adenocarcinomas. Despite its close correlation with the disease, it is unclear whether delta-catenin presents the potential in prostate cancer screening because it is an intracellular protein. In this study, we investigated the hypothesis of delta-catenin accumulation in the urine of prostate cancer patients and its potential pathways of excretion into extracellular milieu. METHODS. Prostate cancer cell cultures, human tissue biopsies, and voided urines were characterized to determine extracellular delta-catenin accumulation and co-isolation with exosomes/prostasomes. RESULTS. We identified delta-catenin in culture media and in the stroma of human prostate cancer tissues. In PC-3 cells in culture, delta-catenin was partially co-localized and co-isolated with raft-associated membrane protein caveolin-1 and glycosylphosphatidylinositol-anchored protein CD59, suggesting its potential excretion into extracellular milieu through exosome/prostasome associated pathways. Interference with endocytic pathway using wortmannin did not block prostasome excretion, but delta-catenin overexpression promoted the extracellular accumulation of caveolin-1. delta-Catenin, caveolin-1, and CD59 were all detected in cell-free human voided urine prostasomes. delta-Catenin immunoreactivity was significantly increased in the urine of prostate cancer patients (P < 0.0005). CONCLUSIONS. This study demonstrated, for the first time, the extracellular accumulation of delta-catenin in urine supporting its potential utility for non-invasive prostate cancer detection. Prostate 69: 411-418, 2009. (C) 2008 Wiley-Liss, Inc.

Maraldo, D., et al. (2007). "Method for quantification of a prostate cancer biomarker in urine without sample preparation." Analytical Chemistry 79(20): 7683-7690.

We describe a macrocantilever-based method for detecting a prostate cancer biomarker (alpha-methylacyl-CoA racemase; AMACR) directly in patient urine without a sample preparation step and without the use of labeled reagents. Clean catch voided urine specimens were prospectively collected from five confirmed prostate cancer patients 3 weeks postbiopsy. The presence of AMACR was measured in a blinded manner by exposing 3 mL of urine to the antiAMACR-immobilized piezoelectric-excited millimeter-sized (PEMC) sensor. The resonance frequency of PEMC decreases as AMACR from sample binds to the antibody on the sensor. The resonance frequency changes for the five patients tested were 4,1314 +/not superset of 35 (n = 2), 269 +/not superset of 17 (n = 2), 977 +/not superset of 64 (n = 3), 600 +/not superset of 31 (n = 2), and 801 +/not superset of 81 (n = 2) Hz, respectively. Positive detection was observed within similar to 15 min. The responses to positive, negative, and buffer controls were -9 +/not superset of 13, -34 +/not superset of 18, and -6 +/not superset of 18 Hz, respectively. Positive verification of AMACR attachment was confirmed by low-pH buffer release. The sensor response was quantitatively related to AMACR concentration in control urine, and the relationship was used in developing an in situ calibration method for quantifying AMACR in patient urine. Estimated concentrations of 42, 2, and 3 fg/mL AMACR were calculated for the three patients' urine, while absence of AMACR was confirmed in control urine (n = 13). Because of simplicity of measurement combined. with high sensitivity and specificity, the method may be a useful adjunct in a point-of-care setting to identify men at increased risk for prostate cancer.

Marszall, M. P., et al. (2015). "Engrailed-2 protein as a potential urinary prostate cancer biomarker: a comparison study before and after digital rectal examination." European Journal of Cancer Prevention 24(1): 51-56.

This study was designed to compare and evaluate the presence of engrailed-2 (EN2) protein in urine collected before and after prostate massage as a diagnostic marker for prostate cancer (PCa). We analysed and compared 76 urine samples (38 before and 38 after prostate massage) from the benign group (BPH) and 66 urine samples (33 before and 33 after prostate massage) from patients with PCa confirmed by prostate biopsy. EN2 levels from the PCa and men with BPH (age range 50-82) were related to the tumour stage, Gleason score and prostate-specific antigen. EN2 levels were determined by enzyme-linked immunosorbent assay in urine. The median EN2 levels in urine after prostate massage were significantly different from those determined in urine before prostate massage (1.25ng/ml in the PCa group and 0.34ng/ml in the BPH). The mean EN2 levels in PCa patients were 3.76-fold higher than those in non-PCa patients after prostate massage. The distinct influence of prostate massage on EN2 levels was found to be related to the Gleason score and tumour stage. EN2 may be considered a marker of PCa with certain limitations, such as those related to tumour staging. The specificity and sensitivity of the protocol are highly dependent on prostate massage. (C) 2014 Wolters Kluwer Health vertical bar Lippincott Williams & Wilkins.

M'Koma, A. E., et al. (2007). "Detection of pre-neoplastic and neoplastic prostate disease by MADI profiling of urine." Biochemical and Biophysical Research Communications 353(3): 829-834.

The heterogeneous progression to the development of prostate cancer (PCa) has precluded effective early detection screens. Existing prostate cancer screening paradigms have relatively poor specificity for cancer relative to other prostate diseases, commonly benign prostatic hyperplasia (BPH). A method for discrimination of BPH, HGPIN, and PCa urine proteome was developed through testing 407 patient samples using matrix assisted laser desorption-mass spectrometry time of flight (MALDI-TOF). Urine samples were adsorbed to reverse phase resin, washed, and the eluant spotted directly for MALDI-TOF analysis of peptides. The processing resolved over 130 verifiable signals of a mass range of 1000-5000 m/z to suggest 71.2% specificity and 67.4% sensitivity in discriminating PCa vs. BPH. Comparing BPH and HGPIN resulted in 73.6% specificity and 69.2% sensitivity. Comparing PCa and HGPIN resulted in 80.8% specificity and 81.0% sensitivity. The high throughput, low-cost assay method developed is amenable for large patient numbers required for supporting biomarker identification. (c) 2006 Elsevier Inc. All rights reserved.

Morgan, R., et al. (2011). "Engrailed-2 (EN2): A Tumor Specific Urinary Biomarker for the Early Diagnosis of Prostate Cancer." Clinical Cancer Research 17(5): 1090-1098.

Purpose: Prostate cancer (PC) is the second most common cause of cancer related death in men. A number of key limitations with prostate specific antigen (PSA), currently the standard detection test, has justified evaluation of new biomarkers. We have assessed the diagnostic potential of Engrailed-2 (EN2) protein, a homeodomain-containing transcription factor expressed in PC cell lines and secreted into the urine by PC in men. Experimental Design: EN2 expression in PC cell lines and prostate cancer tissue was determined by semi-quantative RT-PCR and immunohistochemistry. First pass urine [without prior digital rectal examination (DRE)] was collected from men presenting with urinary symptoms (referred to exclude/confirm the presence of prostate cancer) and from controls. EN2 protein was measured by ELISA in urine from men with PC (n = 82) and controls (n = 102). Results: EN2 was expressed and secreted by PC cell lines and PC tissue but not by normal prostate tissue or stroma. The presence of EN2 in urine was highly predictive of PC, with a sensitivity of 66% and a specificity of 88.2%, without requirement for DRE. There was no correlation with PSA levels. These results were confirmed independently by a second academic center. Conclusions: Urinary EN2 is a highly specific and sensitive candidate biomarker of prostate cancer. A larger multicenter study to further evaluate the diagnostic potential of EN2 is justified. Clin Cancer Res; 17(5); 1090-8. (C)2011 AACR.

Morrissey, J. J., et al. (2015). "Urine Aquaporin 1 and Perilipin 2 Differentiate Renal Carcinomas From Other Imaged Renal Masses and Bladder and Prostate Cancer." Mayo Clinic Proceedings 90(1): 35-42.

Objective: To evaluate the sensitivity and specificity of urine aquaporin 1 (AQP1) and perilipin 2 (PLIN2) concentrations to diagnose clear cell or papillary renal cell carcinoma (RCC) by comparing urine concentrations of these unique biomarkers in patients with RCC, noncancer renal masses, bladder cancer, and prostate cancer. Methods: From February 1, 2012, through October 31, 2012, preoperative urine samples were obtained from patients with a presumptive diagnosis of RCC based on an imaged renal mass, prostate cancer, or transitional cell bladder cancer. Imaged renal masses were diagnosed postnephrectomy-as malignant or benign-by histology. Urine AQP1 and PLIN2 concentrations were measured by using a sensitive and specific Western blot and normalized to urine creatinine concentration. Results: Median concentrations of urine AQP1 and PLIN2 in patients with clear cell and papillary RCC (n=47) were 29 and 36 relative absorbance units/mg urine creatinine, respectively. In contrast, median concentrations in patients with bladder cancer (n=22) and prostate cancer (n=27), patients with chromophobe tumors (n=7), and patients with benign renal oncocytomas (n=9) and angiomyolipomas (n=7) were all less than 10 relative absorbance units/mg urine creatinine (Kruskal-Wallis test, P<.001 vs RCC for both biomarkers) and comparable with those in healthy controls. The area under the receiver operating characteristic curve ranged from 0.99 to 1.00 for both biomarkers. Conclusion: These results support the specificity and sensitivity of urine AQP1 and PLIN2 concentrations for RCC. These novel tumor-specific proteins have high clinical validity and high potential as specific screening biomarkers for clear cell and papillary RCC as well as in the differential diagnosis of imaged renal masses.

Mueller, H., et al. (2008). "Evaluation of serum and urinary myeloid related protein-14 as a marker for early detection of prostate cancer." Journal of Urology 180(4): 1309-1312.

Purpose: Early detection of prostate cancer by prostate specific antigen testing is subject to ongoing controversy. Thus, practical tests to improve or replace prostate specific antigen would be highly desirable. In diagnostic studies promising results were shown for myeloid related protein-14 in serum and urine. However, confirmation in longitudinal population based studies is needed. Materials and Methods: Incident prostate cancer cases (32) and controls (74) matched by age were identified during a 2-year followup of a longitudinal study. The group of cases was further complemented by a sample of 24 prostate cancer cases recruited before initiation of treatment from a clinical study. A commercially available test was used to analyze serum and urinary myeloid related protein-14 in blinded fashion. Results: In contrast to prostate specific antigen, serum and urinary myeloid related protein-14 could not significantly discriminate between prostate cancer cases and controls. Conclusions: In our study, neither serum nor urinary myeloid related protein-14 proved suitable to distinguish prostate cancer cases from controls. Overall myeloid related protein-14 performed much worse than prostate specific antigen and it does not seem useful to reduce false-positive findings of prostate specific antigen in the controversial range of 4 to 10 ng/ml.

Nakayama, K., et al. (2014). "The C-Terminal Fragment of Prostate-Specific Antigen, a 2331 Da Peptide, as a New Urinary Pathognomonic Biomarker Candidate for Diagnosing Prostate Cancer." PloS one 9(9).

Background and Objectives: Prostate cancer (PCa) is one of the most common cancers and leading cause of cancer-related deaths in men. Mass screening has been carried out since the 1990s using prostate-specific antigen (PSA) levels in the serum as a PCa biomarker. However, although PSA is an excellent organ-specific marker, it is not a cancer-specific marker. Therefore, the aim of this study was to discover new biomarkers for the diagnosis of PCa. Materials and Methods: We focused on urine samples voided following prostate massage (digital rectal examination [DRE]) and conducted a peptidomic analysis of these samples using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MSn). Urinary biomaterials were concentrated and desalted using CM-Sepharose prior to the following analyses being performed by MALDI-TOF/MSn: 1) differential analyses of mass spectra; 2) determination of amino acid sequences; and 3) quantitative analyses using a stable isotope-labeled internal standard. Results: Multivariate analysis of the MALDI-TOF/MS mass spectra of urinary extracts revealed a 2331 Da peptide in urine samples following DRE. This peptide was identified as a C-terminal PSA fragment composed of 19 amino acid residues. Moreover, quantitative analysis of the relationship between isotope-labeled synthetic and intact peptides using MALDI-TOF/MS revealed that this peptide may be a new pathognomonic biomarker candidate that can differentiate PCa patients from non-cancer subjects. Conclusion: The results of the present study indicate that the 2331 Da peptide fragment of PSA may become

a new pathognomonic biomarker for the diagnosis of PCa. A further large-scale investigation is currently underway to assess the possibility of using this peptide in the early detection of PCa.

Okamoto, A., et al. (2009). "Protein profiling of post-prostatic massage urine specimens by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry to discriminate between prostate cancer and benign lesions." Oncology Reports 21(1): 73-79.

Post-prostatic massage urine specimens (PMUS) are expected to be rich in proteins originating from the prostatic acini. In this study, we created a PMUS bank consisting of 57 samples obtained from patients with biopsy-proven prostate cancer (PC) and 56 samples from subjects with biopsy-proven benign lesions to analyze protein profiles of PMUS by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS), Strong anion-exchange (Q10), weak cation-exchange (CM10) and immobilized metal affinity capture (IMAC30) ProteinChip Arrays were used for protein profiling. In PC samples, single-marker analysis detected 49 mass peaks that were significantly up-regulated and 23 peaks that were significantly down-regulated, compared with peaks obtained from benign lesion samples. To confirm reproducibility we performed additional three rounds of assay using CM10 chip with pH 4.0 binding buffer. Among these significant peaks, a peak of m/z 10788 was significant throughout all 4 rounds of assays. For hierarchical clustering analysis (HCA), we used the 72 peaks which revealed significant differences in single-marker analysis. The heat map discriminated PC from benign lesions with a sensitivity of 91.7% and a specificity of 83.3%. Therefore, SELDI-TOF MS profiling of PMUS can be applied to differentiate patients with PC from cancer-free subjects. However, further investigation is required to verify the usefulness of this method in clinical practice.

Pandha, H., et al. (2012). "Urinary engrailed-2 (EN2) levels predict tumour volume in men undergoing radical prostatectomy for prostate cancer." Bju International 110(6B): E287-E292.

What's known on the subject? and What does the study add? There are a lot of potential prostate cancer biomarkers being evaluated. All aim to improve on the sensitivity and specificity of PSA. EN2 was recently shown by our group to have better sensitivity and specificity than PSA. EN2 is a simple ELISA test and is not dependent on other parameters, even PSA, unlike all the other current biomarkers under evaluation. To date, no marker correlates with the amount of cancer present - the present study shows this positive correlation with EN2 in men undergoing prostatectomy. The potential utility of this work is that by knowing that the level of EN2 corresponds to the amount of cancer present, irrelevant of tumour grade and number of cancer foci, we can define an EN2 level corresponding to small cancers, which can then undergo surveillance. We are conducting a further study that is aimed at determining whether the levels of EN2 in urine can indicate 'significant' vs 'non-significant cancer' using the threshold of 0.5 mL cancer (after Epstein's work). OBJECTIVES To evaluate the relationship between levels of a recently described prostate cancer biomarker engrailed-2 (EN2) in urine and cancer volume in men who had undergone radical prostatectomy (RP) for prostate cancer. To date, prostate-specific antigen (PSA) levels have not reliably predicted prostate cancer volume. Reliable volume indicator biomarker(s) may aid management decisions, e. g. active treatment vs active surveillance. PATIENTS AND METHODS Archived patient samples from the Aarhus Prostate Cancer Project, Denmark, were assessed. Pre-treatment mid-stream urines, without preceding prostatic massage, were collected and stored at -80 degrees C. Urinary EN2 levels were measured by a recently published enzyme-linked immunosorbent assay. RESULTS In all, 88 of the whole cohort of 125 men (70%) were positive for EN2 in their urine (> 42.5 g/L); 38/58 (65%) men where cancer volume data was available. There was no statistical relationship between urinary EN2 levels and serum PSA levels. PSA levels did not correlate with tumour stage, combined Gleason grade, total prostatic weight or cancer volume. There was a strong statistical relationship between urinary EN2 and prostate cancer volume by linear regression (P = 0.006). Higher EN2 levels correlated with tumour stage T1 vs T2 (P = 0.027). CONCLUSIONS Pre-surgical urinary EN2 levels were associated with increasing tumour stage and closely reflected the volume of cancer in RP specimens. Given the ease of collection (no prostatic massage required) and the simplicity, low cost and robustness of the assay, EN2 may become a useful biomarker in not only identifying which patients have prostate cancer but may also facilitate risk stratification by indicating the burden of tumour volume.

Prager, A. J., et al. (2013). "Urinary aHGF, IGFBP3 and OPN as diagnostic and prognostic biomarkers for prostate cancer." Biomarkers in Medicine 7(6): 831-841.

Aim: Serum PSA screening for prostate cancer (PCa) is controversial. Here, we identify three urinary biomarkers - aHGF, IGFBP3 and OPN - for PCa screening and prognostication. Methods: Urinary aHGF, OPN and IGFBP3 from healthy men (n = 19) and men with localized (n = 65) and metastatic (n = 36) PCa were quantified via ELISA. Mann-Whitney nonparametric t-test and the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) analyses were used to analyze associations. Results: Mean aHGF and IGFBP3 levels were significantly elevated in PCa patients versus controls (p = 0.0006 and p = 0.0012, respectively), and the area under the curve of the receiver operating characteristic curve (indicator of diagnostic accuracy) for aHGF and IGFBP3 was 0.75 and 0.74, respectively. OPN levels were significantly higher in metastatic groups (p = 0.0060) versus localized and controls (area under the curve = 0.68). Conclusion: Urinary aHGF and IGFBP3 exhibit the capacity for diagnostic discrimination for PCa, whereas OPN may indicate presence of metastatic disease.

Principe, S., et al. (2012). "Identification of Prostate-Enriched Proteins by In-depth Proteomic Analyses of Expressed Prostatic Secretions in Urine." Journal of Proteome Research 11(4): 2386-2396.

Urinary expressed prostatic secretion or "EPS-urine" is proximal tissue fluid that is collected after a digital rectal exam (DRE). EPS-urine is a rich source of prostate-derived proteins that can be used for biomarker discovery for prostate cancer (PCa) and other prostatic diseases. We previously conducted a comprehensive proteome analysis of direct expressed prostatic secretions (EPS). In the current study, we defined the proteome of EPS-urine employing Multidimensional Protein Identification Technology (MudPIT) and providing a comprehensive catalogue of this body fluid for future biomarker studies. We identified 1022 unique proteins in a heterogeneous cohort of 11 EPS-urines derived from biopsy negative noncancer diagnoses with some benign prostatic diseases (BPH) and low-grade PCa, representative of secreted prostate and immune system-derived proteins in a urine background. We further applied MudPIT-based proteomics to generate and compare the differential proteome from a subset of pooled urines (pre-DRE) and EPS-urines (post-DRE) from noncancer and PCa patients. The direct proteomic comparison of these highly controlled patient sample pools enabled us to define a list of prostate-enriched proteins detectable in EPS-urine and distinguishable from a complex urine protein background. A combinatorial analysis of both proteomics data sets and systematic integration with publicly available proteomics data of related body fluids, human tissue transcriptomic data, and immunohistochemistry images from the Human Protein Atlas database allowed us to demarcate a robust panel of 49 prostate-derived proteins in EPS-urine. Finally, we validated the expression of seven of these proteins using Western blotting, supporting the likelihood that they originate from the prostate. The definition of these prostatic proteins in EPS-urine samples provides a reference for future investigations for prostatic-disease biomarker studies.

Russo, A. L., et al. (2009). "Urine Analysis and Protein Networking Identify Met as a Marker of Metastatic Prostate Cancer." Clinical Cancer Research 15(13): 4292-4298.

Purpose: Metastatic prostate cancer is a major cause of death of men in the United States. Expression of met, a receptor tyrosine kinase, has been associated with progression of prostate cancer. Experimental Design: To investigate met as a biomarker of disease progression, urinary met was evaluated via ELISA in men with localized (n = 75) and metastatic (n = 81) prostate cancer. Boxplot analysis was used to compare the distribution of met values between each group. We estimated a receiver operating characteristic curve and the associated area under the curve to summarize the diagnostic accuracy of met for distinguishing between localized and metastatic disease. Protein-protein interaction networking via yeast two-hybrid technology supplemented by Ingenuity Pathway Analysis and Human Interactome was used to elucidate proteins and pathways related to met that may contribute to progression of disease. Results: Met distribution was significantly different between the metastatic group and the group with localized prostate cancer and people with no evidence of cancer (P < 0.0001). The area under the curve for localized and metastatic disease was 0.90, with a 95% confidence interval of 0.84 to 0.95. Yeast two-hybrid technology, Ingenuity Pathway Analysis, and Human Interactome identified 89 proteins that interact with met, of which 40 have previously been associated with metastatic prostate cancer. Conclusion: Urinary met may provide a noninvasive biomarker indicative of metastatic prostate cancer and may be a central regulator of multiple pathways involved in prostate cancer progression.

Sardana, G. and E. P. Diamandis (2009). "The kallikrein family of proteins as urinary biomarkers for the detection of prostate cancer." Clinical Biochemistry 42(13-14): 1483-1486.

Background: Several urinary biomarkers have been assessed as showing a discriminatory ability to differentially diagnose prostate cancer, albeit with manipulation of the prostate. Here we examine the clinical utility of multiple members of the kallikrein family of proteins in non-manipulative urinary biomarker testing. Methods: Forty urine samples were collected from patients admitted for urological examination. Twenty, with a confirmed benign diagnosis and 20 with prostate cancer. The levels of 14 kallikrein proteins were measured in patient's urine and normalized for creatinine. Results: Ten of the 14 kallikreins tested had detectable levels in urine. However, none showed statistical significance in discriminating patients. Serum PSA was superior to urine PSA and other urinary kallikreins in separating patients with and without prostate cancer. Conclusions: We were unable to distinguish men with and without prostate cancer using multiple kallikreins as urinary biomarkers. These results highlight the difficulties in diagnosing prostate cancer via urine testing for soluble biomarkers. (C) 2009 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

Schiffer, E., et al. (2012). "Urinary proteome analysis for prostate cancer diagnosis: Cost-effective application in routine clinical practice in Germany." International Journal of Urology 19(2): 118-125.

Objectives: Capillary electrophoresis mass spectrometry urinary proteome analysis for prostate cancer has been shown to be highly accurate in the detection of prostate cancer. The aim of the present study was to report our experience with routine application of this test in clinical practice and its cost-effectiveness. Methods: The urinary proteome analysis for prostate cancer test was carried out in 211 patients in outpatient centers. In 184 of them, data about their followup and the test results were available for analysis. Prostate cancer was detected in 49 cases. Results: The test correctly recognized 42 out of 49 tumor patients, showing a sensitivity of 86% (95% confidence interval 73-94). Of 135 prostate cancer-negative patients, 79 had a negative urinary proteome analysis for prostate cancer test (specificity 59% [79/135 95% confidence interval 50-66]). Negative and positive predictive values were 92% (95% confidence interval 84-96) and 43% (95% confidence interval 33-53), respectively. A statistically significant (P < 0.0005) improvement in terms of diagnostic accuracy was observed in comparison with serum prostate-specific antigen and percent-free prostate-specific antigen. Whereas the urinary proteome analysis for prostate cancer test results agreed in 65.7% with follow-up reference results, prostate-specific antigen achieved 33.3% and percent-free prostate-specific antigen achieved 42.7%. Cost-effectiveness analysis showed that the urinary proteome analysis for prostate cancer strategy outperformed the biopsy approach as well as prostate-specific antigen tests. Conclusions: The non-invasive urinary proteome analysis for prostate cancer test appears to be a helpful addition to prostate cancer diagnostics for patients with suspicious prostate-specific antigen and/or digital-rectal examination.

Stovsky, M., et al. (2011). "Prostate-specific Antigen/Solvent Interaction Analysis: A Preliminary Evaluation of a New Assay Concept for Detecting Prostate Cancer Using Urinary Samples." Urology 78(3): 601-605.

OBJECTIVE To provide preliminary clinical performance evaluation of a novel prostate cancer (CaP) assay, prostate-specific antigen/solvent interaction analysis (PSA/SIA) that focused on changes to the structure of PSA. METHODS Two-hundred twenty-two men undergoing prostate biopsy for accepted clinical criteria at 3 sites (University Hospitals Case Medical Center in Cleveland, Cleveland Clinic, and Veterans Administration Boston Healthcare System) were enrolled in institutional review board-approved study. Before transrectal ultrasound-guided biopsy, patients received digital rectal examination with systematic prostate massage followed by collection of urine. The PSA/SIA assay determined the relative partitioning of heterogeneous PSA isoform populations in urine between 2 aqueous phases. A structural index, K, whose numerical value is defined as the ratio of the concentration of all PSA isoforms, was determined by total PSA enzyme-linked immunosorbent assay and used to set a diagnostic threshold for CaP. Performance was assessed using receiver operating characteristic (ROC) analysis with biopsy as the gold standard. RESULTS Biopsies were pathologically classified as case (malignant, n = 100) or control (benign, n = 122). ROC performance demonstrated area under the curve = 0.90 for PSA/SIA and 0.58 for serum total PSA. At a cutoff value of k = 1.73, PSA/SIA displayed sensitivity = 100%, specificity = 80.3%, positive predictive value = 80.6%, and negative predictive value = 100%. No attempt was made in this preliminary study to further control patient population or selection criteria for biopsy, nor did we analytically investigate the type of structural differences in PSA that led to changes in k value. CONCLUSION PSA/SIA provides ratiometric information independently of PSA concentration. In this preliminary study, analysis of the overall structurally heterogeneous PSA isoform population using the SIA assay showed promising results to be further evaluated in future studies. UROLOGY 78: 601-606, 2011. (C) 2011 Elsevier Inc. All rights reserved.

True, L. D., et al. (2010). "CD90/THY1 is overexpressed in prostate cancer-associated fibroblasts and could serve as a cancer biomarker." Modern Pathology 23(10): 1346-1356.

A by-product in the processing of prostate tissue for cell sorting by collagenase digestion is the media supernatant that remains after the cells are harvested. These supernatants contain proteins made by the cells within the tissue. Quantitative proteomic analysis of N-glycosylated proteins detected an increased amount of CD90/THY1 in cancer supernatants compared with non-cancer supernatants. Immunohistochemistry showed that in all carcinomas, regardless of Gleason grade, a layer of CD90-positive stromal fibroblastic cells, similar to 5 to 10 cells deep, was localized to tumor glands. In contrast, a no more than 1-cell wide girth of CD90-positive stromal cells was found around benign glands. The increased number of CD90-positive stromal cells in cancer correlated with overexpression of CD90 mRNA detected by gene expression analysis of stromal cells obtained by laser-capture microdissection. There is increasing evidence that cancer-associated stroma has a function in both tumor progression and carcinogenesis. Most experiments to identify cancer biomarkers have focused on the cancer cells. CD90, being a marker for prostate cancer-associated stroma, might be a potential biomarker for this cancer. A non-invasive test could be provided by a urine test. Proteomic analysis of urine from patients with prostate cancer identified CD90; conversely, CD90 was not detected in the urine of post-prostatectomy patients. Furthermore, this urinary CD90 protein was a variant CD90 protein not known to be expressed by such cells as lymphocytes that express CD90. These CD90 results were obtained from similar to 90 cases consisting of proteomic analysis of tissue and urine, immunohistochemistry, western blot analysis of tissue media, flow cytometry of cells from digested tissue, and reverse transcriptase polymerase chain reaction analysis of isolated stromal cells. Modern Pathology (2010) 23, 1346-1356; doi:10.1038/modpathol.2010.122; published online 18 June 2010

Valmu, L., et al. (2010). "Proteomic analysis of pancreatic secretory trypsin inhibitor/tumor-associated trypsin inhibitor from urine of patients with pancreatitis or prostate cancer." Methods in molecular biology (Clifton, N.J.) 641: 347-357.

The development of proteomic methods, especially mass spectrometry, has brought new possibilities to tumor marker research. Pancreatic secretory trypsin inhibitor (PSTI), a common known biomarker for various malignancies, occurs on genetic variants that we are able to detect at the protein level with proteomic techniques using immunoaffinity capture prior to liquid chromatography-mass spectrometry (LC-MS). We also show that PSTI can be detected in urine from cancer patients using a two-step peptide enrichment technique and LC-MS. These results show that tumor-associated peptides can be detected in urine by proteomic techniques.

Varambally, S., et al. (2008). "Golgi Protein GOLM1 Is a Tissue and Urine Biomarker of Prostate Cancer." Neoplasia 10(11): 1285-U1104.

Prostate cancer is the most common type of tumor found in American men and is the second leading cause of cancer death in males. To identify biomarkers that distinguish prostate cancer from normal, we compared multiple gene expression profiling studies. Through meta-analysis of expression array data from multiple prostate cancer studies, we identified GOLM1 (Golgi membrane protein 1, Golm 1) as consistently up-regulated in clinically localized prostate cancer. This observation was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) and validated at the protein level by immunoblot assay and immunohistochemistry. Prostate epithelial cells were identified as the cellular source of GOLM1 expression using laser capture microdissection. Immunohistochemical staining localized the GOLM1 signal to the subapical cytoplasmic region, typical of a Golgi distribution. Surprisingly

Vermassen, T., et al. (2015). "Urinary Prostate Protein Glycosylation Profiling as a Diagnostic Biomarker for Prostate Cancer." Prostate 75(3): 314-322.

BACKGROUND. Serum prostate-specific antigen (sPSA) measurement is widely used as opportunistic screening tool for prostate cancer (PCa). sPSA suffers from considerable sensitivity and specificity problems, particularly in the diagnostic gray zone (sPSA 4-10 mu g/L). Furthermore, sPSA is not able to discriminate between poorly-, moderately-, and well-differentiated PCa. We investigated prostatic protein glycosylation profiles as a potential PCa biomarker. METHODS. Differences in total urine N-glycosylation profile of prostatic proteins were determined between healthy volunteers (n = 54), patients with benign prostate hyperplasia (BPH; n = 93) and newly diagnosed PCa patients (n = 74). Variations in N-glycosylation profile and prostate volume were combined into one urinary glycoprofile marker (UGM). Additionally, differences in N-glycosylation were identified between Gleason <7, = 7, and >7. RESULTS. The UGM was able to discriminate BPH from PCa, overall and in

the diagnostic gray zone (P < 0.001). The UGM showed comparable diagnostic accuracy to sPSA, but gave an additive diagnostic value to sPSA (P < 0.001). In the diagnostic gray zone the UGM performed significantly better than sPSA (P < 0.001). A significant difference was found in core-fucosylation of biantennary structures and overall core-fucosylation of multiantennary structures between Gleason < 7 and Gleason > 7 (P = 0.010 and P = 0.020, respectively) and between Gleason = 7 and Gleason >7 (P = 0.011 and P = 0.025, respectively). CONCLUSIONS. The UGM shows high potential as PCa biomarker, particularly in the diagnostic gray zone. Further research is needed to validate these findings. (C) 2014 Wiley Periodicals, Inc.

Vermassen, T., et al. (2014). "Capillary electrophoresis of urinary prostate glycoproteins assists in the diagnosis of prostate cancer." Electrophoresis 35(7): 1017-1024.

Prostate marker assays are widely used for detection of prostate cancer (PCa) but are associated with considerable sensitivity and specificity problems. Therefore, we investigated prostatic protein glycosylation profiles as a potential biomarker. We determined the urinary asparagine-linked glycan (N-glycan) profile of prostatic proteins of healthy volunteers (n = 25), patients with benign prostate hyperplasia (BPH; n = 62) and newly diagnosed PCa patients (n = 42) using DNA-sequencer-assisted fluorophore-assisted carbohydrate electrophoresis. Through squeezing of the prostate, a sufficient amount of prostatic proteins was obtained for direct structural analyses of N-glycan structures. N-glycans of PCa compared to BPH were characterized by a significant decrease in triantennary structures (p = 0.047) and overall fucosylation (p = 0.026). Prostate-specific antigen (PSA) and the urinary glycoprofile marker showed comparable overall receiver operating characteristic curve analysis as well as in the diagnostic gray zone with serum PSA values between 4 and 10g/L. However, when combining PSA and the urinary glycoprofile marker, the latter gave an additive diagnostic value to serum PSA (p 0.001). In conclusion, N-glycosylation profiling demonstrated differences between BPH and PCa. These changes could lead to the discovery of a new biomarker for PCa.

Wayner, E. A., et al. (2012). "Development of an ELISA to detect the secreted prostate cancer biomarker AGR2 in voided urine." Prostate 72(9): 1023-1034.

BACKGROUND Comparative transcriptomics between sorted cells identified AGR2 as one of the highest up-regulated genes in cancer. Overexpression in primary tumors was verified by tissue microarray analysis. AGR2 encodes a 19-kDa secreted protein that might be found in urine. METHODS Monoclonal antibodies were generated against AGR2. One antibody pair, P1G4 (IgG1) to capture and P3A5 (IgG2a) to detect, showed good performance characteristics in a sandwich ELISA. This assay could detect AGR2 at sub ng/ml quantities. RESULTS AGR2 was detected in tissue digestion media of tumor specimens and culture media of AGR2-secreting prostate cancer cell lines. Additional testings involved frozen section immunohistochemistry, immunoprecipitation, and Western blot analysis. Voided urine samples were collected from pre-operative cancer patients, and urinary protein was desalted and concentrated by filtration. The amount of AGR2 detected was scored as pg/100?mu g total protein, and then converted to pg/ml urine. The developed ELISA detected AGR2 protein, ranging from 3.6 to 181?pg/ml, in an initial cohort of samples. AGR2 was not detected in the urine of non-cancer and a bladder cancer patient. CONCLUSIONS For prostate cancer, an AGR2 urine test could be used for diagnosis. The data, although derived from a small number of samples assayed, showed that developing such a test for clinical application is viable because AGR2 is specific to cancer cells, and apparently secreted into urine. Prostate 72:10231034, 2012. (c) 2011 Wiley Periodicals, Inc.

Whitaker, H. C., et al. (2010). "The rs10993994 Risk Allele for Prostate Cancer Results in Clinically Relevant Changes in Microseminoprotein-Beta Expression in Tissue and Urine." PloS one 5(10).

Background: Microseminoprotein-beta (MSMB) regulates apoptosis and using genome-wide association studies the rs10993994 single nucleotide polymorphism in the MSMB promoter has been linked to an increased risk of developing prostate cancer. The promoter location of the risk allele, and its ability to reduce promoter activity, suggested that the rs10993994 risk allele could result in lowered MSMB in benign tissue leading to increased prostate cancer risk. Methodology/Principal Findings: MSMB expression in benign and malignant prostate tissue was examined using immunohistochemistry and compared with the rs10993994 genotype. Urinary MSMB concentrations were determined by ELISA and correlated with urinary PSA, the presence or absence of cancer, rs10993994 genotype and age of onset. MSMB levels in prostate tissue and urine were greatly reduced with tumourigenesis. Urinary MSMB was better than urinary PSA at differentiating men with prostate cancer at all

Gleason grades. The high risk allele was associated with heterogeneity of MSMB staining and loss of MSMB in both tissue and urine in benign prostate. Conclusions: These data show that some high risk alleles discovered using genome-wide association studies produce phenotypic effects with potential clinical utility. We provide the first link between a low penetrance polymorphism for prostate cancer and a potential test in human tissue and bodily fluids. There is potential to develop tissue and urinary MSMB for a biomarker of prostate cancer risk, diagnosis and disease monitoring.

Wittke, S., et al. (2007). "Capillary electrophoresis coupled to mass spectrometry for proteome analysis. An innovative diagnostic method for prostate and bladder cancer." Urologe 46(7): 733-739.

We developed a proteomics-based technology for the non-invasive detection of urothelial and prostate carcinoma. Using capillary electrophoresis coupled to mass spectrometry, disease-specific changes in the urinary proteome were detected and subsequently relevant polypeptides were employed as disease-specific biomarkers. Here we report the results of various studies including approximately 1,000 patients with different diseases and healthy volunteers. The results of these studies revealed that prostate and urothelial carcinoma can be detected by using disease-specific polypeptide patterns. Preliminary results also indicate that the tumour stage of an urothelial carcinoma can be estimated by this approach. In conclusion, this new and non-invasive application might help to improve the diagnostic methods already available.