Urinary markers for prostate cancer

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Prostate cancer is the commonest solid-organ malignancy to affect men in Europe and the USA; it is estimated that one in six men will develop this cancer in their lifetime. Current screening relies on a digital rectal examination with a serum prostate-specific antigen test. Novel urinary diagnostic tests are potentially interesting screening tools for this disease. We examined published reports assessing the use of urinary markers for the diagnosis of prostate cancer. Using a PubMed-based search we identified studies of urinary markers for prostate cancer published from 1985 to February 2006 using the search terms ‘urine’, ‘marker’ and ‘prostate cancer’. Studies to date have used small cohorts and relied on prostatic biopsies to provide histology. The sensitivity and specificity of markers are wide ranging but with only a few studies published on each putative marker it is difficult to assess their potential impact. Using urinary biomarkers for prostate cancer is a relatively novel diagnostic approach; they are appealing as a screening test because they are not invasive. Further work is needed to identify and validate ‘signature markers’ indicative of prostatic malignancy. The newer proteomic platforms are promising biomarker discovery tools that might uncover the next generation of urinary biomarkers.

KEYWORDS
urine, marker, prostate cancer, proteomics

INTRODUCTION

Prostate cancer is the commonest solid-organ malignancy diagnosed in men in Europe and the USA; it is the second most frequent cause of cancer-related death in men. An estimated 200 000 men are diagnosed with it in the USA every year, with ≈30 000 deaths annually [1]. The indolent nature of early prostate cancer makes it amenable to curative therapy and this in turn renders it a suitable disease for which to pursue a successful screening programme. Since the introduction of PSA screening the incidence of prostate cancer has increased dramatically and it is now estimated that 80% of men in their eighth decade have invasive prostate cancer. There has been a 30% increase in the number of men diagnosed with the malignancy over the last 25 years [2], with predictions that the incidence could double by the year 2030 [3]. Currently it is not possible to differentiate between clinically relevant prostate cancer and that which requires no further intervention. For this reason, if screening for this disease is to continue, better molecular markers that can make this distinction are urgently required. This in turn will reduce unnecessary biopsies and select patients suitable for appropriate therapy.

THE DIAGNOSIS OF PROSTATE CANCER

The current screening method relies on a combination of PSA and a DRE with subsequent TRUS and biopsy if there is suspicion of cancer. It is well recognized that the serum PSA level as a biomarker of prostate cancer is imperfect, in that it does not always suggest the presence of cancer and conversely will rise in the presence of BPH, prostatitis and after urethral manipulation [4]. The combination of a DRE, PSA and TRUS and biopsy will diagnose ≈40% of men with localized cancer when they actually have extraprostatic spread [5]. At a serum PSA level of 4 ng/mL there is a ≈20% risk of prostate cancer, and at >10 ng/mL it is 42–64% [6]. Conversely, some men with a PSA level of <4 ng/mL might have prostate cancer (estimated at 20–30%), resulting in undiagnosed disease [7]. Various concepts, such as free-to-total PSA ratio [8], PSA velocity, PSA density [9] and age-specific PSA ranges [10] have been introduced, but their clinical significance is still under investigation. In addition to these, PSA isoforms are also being investigated in terms of their ability to improve the diagnosis of prostate cancer.

Urine represents an interesting fluid in which to seek biomarkers; it is readily available and obtainable noninvasively and it can be used to detect either exfoliated cancer cells or secreted prostatic products that could indicate the presence of prostate cancer. Furthermore, as prostatic products are released directly into the genitourinary tract, it might represent an appropriate site for the early detection of prostatic malignancy. DNA, RNA and protein markers have all been proposed as suitable diagnostic agents. The purpose of the present article is to review published reports and summarize the wide variety of urine-based diagnostic tests for prostate cancer.

METHODS

The PubMed database was searched for articles using the terms ‘urine’, ‘marker’ and ‘prostate cancer’ in combination, from 1985 up to and including February 2006. Limits were set to only include articles in English and relating to human subjects. Pre-existing review articles were excluded and only original articles with the above-mentioned search terms were included in the analysis. In addition, papers pertaining to markers of bone resorption for prostatic skeletal metastases were not accounted for, as they represent markers of advanced disease and therefore did not fall within the remit of this review. In all, 32 articles were identified using this search strategy.
The data were reviewed for the number of cohorts and controls and the technique used to identify the marker. Details on voided urine vs prostatic massage were noted. The calculated sensitivity and specificity for each marker was recorded, where possible. Among the studies identified it was possible to divide the markers into DNA-, RNA- or protein-based biomarkers. Many of the studies were limited because few patients were included and most based the histology on the Gleason score obtained at biopsy. In addition, for each biomarker there were few papers and hence the results need to be interpreted with caution. The 32 identified reports of prostate cancer urinary biomarkers were grouped according to whether DNA, RNA or protein markers were investigated.

DNA MARKERS

The glutathione-S-transferase P1 gene (GSTP-1) belongs to a family of enzymes involved in protecting DNA from free radicals. Prostate cancer is associated with the loss of GSTP-1 expression due to promoter hypermethylation. This event is the most frequent somatic genome alteration reported in prostate cancer (>90% of cancers) [11] and in high-grade prostate intraepithelial neoplasia (HGPIN, 70%). GSTP-1 represents an attractive biomarker for prostate cancer as it is seldom present in non-cancerous prostate tissue. Methylation markers are detected with methylation specific PCR (MSP); this can be the conventional form using two sets of primers specifically designed to amplify the methylated sequence. Quantitative MSP (QMSP) is a fluorescence based real-time quantitative assay that measures methylated alleles of a single region amongst unmethylated DNA. The results for the detection of GSTP-1 in urine have been disappointing. Goessl et al. [12] reported that 36% of patients with cancer were positive, with a specificity of 100% [21] were assessed using a non-quantitative fluorogenic MSP assay; 45% of patients enrolled in the study had advanced disease). Cairns et al. [13] reported 21.4% positive, with the specificity not determined in 28 patients, using a conventional MSP method. Jeronimo et al. [14] had 30.4% positive and a specificity of 95% with 100 patients using conventional MSP, but using the QMSP assay they had 18.8% positive with a specificity of 93% in 100 patients. Gonzalgo et al. [15] reported 38.9% of men positive but the specificity was not determined, in 39 patients and using conventional MSP.

The low detection rates might be improved by prostatic massage before voiding; Goessl et al. [16] gave a 73% sensitivity in urine voided after massage with a specificity of 98% in 40 men with cancer and 46 controls, using conventional MSP methods. However, Crocitto et al. [17] used a DRE with massage, followed by ‘urethral milking’ before biopsy. They had useable material in 86% of the men (24 with cancer and 34 controls); the sensitivity was 46% and specificity 56% with this method.

Hoque et al. [18] assessed aberrant methylation of nine separate gene promoters (including GSTP-1) in urinary sediment. The study involved 52 patients with prostate cancer and 91 controls; they identified promoter hypermethylation in at least one gene in all cancer samples, but all controls also showed low levels of methylation of five genes. Using a combination of four specific genes (including GSTP-1) they concluded that the sensitivity was 87% and the specificity 100%.

Two studies were identified investigating loss of heterozygosity as a tumour marker. Thuret et al. [19] assessed six locations in 99 men with a PSA level of 4–10 ng/mL before biopsy and found one or more deletions in the prostatic fluid of 57 patients; 58% had prostate cancer. This gave a sensitivity of 86.7% and a specificity of 44%. Cussenot et al. [20] examined four locations in 11 men, with 21 controls, and derived a sensitivity of 73% and a specificity of 67%. Both studies used the first void of urine after massage.

Urinary 8-hydroxydeoxyguanosine (8-OHdG) is considered as a biomarker of generalized cellular oxidative stress and has been linked to many diseases, including cancers. Increased urinary concentrations of 8-OHdG were detected by ELISA in the urine of patients with prostate and bladder cancer [21]. That study included 16 patients with prostate cancer, 15 with bladder cancer and 24 normal controls, and established that the mean values of urinary 8-OHdG were higher in patients than in healthy individuals, with a sensitivity of 31% and specificity of 100%. Cussenot et al. [22] examined mitochondrial DNA markers in early-stage prostate cancer; they identified 20 DNA mutations in tumour tissue from three patients and identical mutations in their plasma and urine. No larger study has been reported since this pilot in 2001.

RNA MARKERS

DD3 (PCA3) is the most prostate cancer-specific gene that has been described to date, with overexpression in >95% of primary prostate cancer specimens and in prostate cancer metastasis [23,24]. DD3 is located on chromosome 9 and found exclusively in the prostate epithelial cells. Reverse transcriptase (RT)-PCR on urine sediments obtained after prostatic massage showed a sensitivity and specificity of DD3 of 86.6% and 89%, respectively [25]. The study by Fradet et al. used a nucleic acid amplification assay to simultaneously detect PSA mRNA as a marker of prostate cells, with DD3 RNA, which is expressed in prostate cancer cells. They used the first voided urine sample after a DRE. In men with a PSA level of <4 ng/mL they found a sensitivity of 74% and a specificity of 91%. They concluded that their urine test would be an important adjunct for the early detection of prostate cancer.

The study by Hessels et al. [26] showed that DD3 is up-regulated 6–1500-fold in prostate tumours compared to adjacent non-neoplastic prostate tissue, with a median up-regulation of 66-fold. In prostate tissues containing <10% cancer cells, there was a median up-regulation of 11-fold. They used a fluorescence-based quantitative RT-PCR to analyse for DD3 on voided urine samples from a group of 108 men after a DRE. The RT-PCR assay is capable of detecting a few malignant cells in a background of predominantly nonmalignant cells, meaning that microdissection is not required. DD3 is a promising potential urinary biomarker for prostate cancer.

Crocitto et al. [17] analysed hTERT RNA expression by RT-PCR in a cohort of 14 patients with cancer and 35 controls; the samples were obtained by the same method as for GSTP-1 analysis. This gave a sensitivity of 36% and a specificity of 66%. Wang et al. [27] assessed survivin mRNA expression in voided urine from patients with bladder, prostate (10 men) and renal cancer, in addition to 20 controls. They used a nested RT-PCR technique and found no sensitivity
with other chromosomes. TERT activity is important in maintaining the telomeric ends of chromosomes. Telomere activity is involved in cell senescence and is critical for the histological diagnosis of prostate cancer. Ziele et al. [28] extracted cellular RNA from prostatic secretions obtained from urine specimens after massage, and RT-PCR was used to detect AMACR. This study had 21 patients enrolled (10 with cancer, two with HGPIN, and nine cancer-free). The quantification of AMACR transcripts normalized to PSA transcripts in prostatic secretions was predictive of prostate cancer, with neither the AMACR nor PSA mRNA levels being predictive of cancer when used alone. The authors also claimed that their results tended to exclude patients with clinically insignificant disease.

Telomerase is coded for by the TERT gene and is important in maintaining the telomeric ends of chromosomes. Telomere activity has been identified in cell senescence and malignancy, capping and protecting the ends of chromosomes. Telomerase activity is detected prostate cancer [29,30]. Botchkina et al. [31], using a quantitative real-time PCR telomeric repeat amplification protocol (TRAP) assay on a group of 56 urine samples from 44 men with BPH, nine with cancer, two with HGPIN, and one with atypical cells, gave 100% sensitivity and 88.6% specificity.

PROTEIN MARKERS

Protein markers of prostate cancer are represented most of the published studies in this review. Secreted proteins or their products can be detected in various ways, e.g. the traditional antibody-based assay approach, or using novel proteomic platforms. These newer strategies allow a ‘high throughput’ approach to searching for biomarkers, with the advantage that they can provide quantitative information.

A protein urinary biomarker reported recently is thymosin β15 (TB-15). Only one paper to date has been published advocating the use of this urinary marker for detecting prostate cancer [32]. The same group developed an ELISA capable of detecting TB-15 protein in the urine [33]. TB-15 has a restricted expression profile and is virtually absent from normal adult tissues, but is up-regulated in malignant human prostate and breast cancer. It is a member of the multiple β thymosin family, which consists of 5 kDa peptides. The expression of TB-15 correlates positively with increased motility and metastatic potential of prostate cancer cell lines [34]. A threshold of 40 ng/mL was determined for urinary levels of TB-15. The authors also found that a combination of both TB-15 and PSA (PSA level of >2.5 ng/mL + TB-15 >40, or a PSA level of ≤2.5 + TB-15 >90) gave a diagnostic sensitivity equal to a PSA threshold of 2.5 ng/mL while improving the specificity from 55.4% to 70.8% in a control group of 130 patients with urological conditions excluding prostate disease. The specificity improved by 15%, from 46.9% to 62.2%, in a control group of 209 patients with urological conditions including prostate disease [32].

The AMACR gene is involved in β-oxidation of branched-chain fatty acids and fatty acid derivatives. The positivity of AMACR in prostate cancer is important, in that the main sources of branched-chain fatty acids in the diet come from beef and dairy products. Various studies have shown that the consumption of these correlates with a greater risk of prostate cancer. Western blot analysis for AMACR was used on voided urine after TRUS and biopsy, showing a 100% sensitivity and 58% specificity for prostate cancer detection in a cohort of 26 men [35]. This group also found AMACR positivity in one of two men with atypia on biopsy; on re-biopsy prostate cancer was detected. It is possible that some of the patients in that study who tested positively for AMACR in their urine but had a negative biopsy might have had occult prostate cancer, which might be identified if further biopsies were taken, thus improving the specificity of the test.

A study by Meid et al. [36] found that telomerase activity detected prostate cancer cells in the urine after prostatic massage, with a sensitivity of 58% and a specificity of 100%. They used a modified TRAP assay on urine from 16 patients who subsequently had a radical prostatectomy; they also used urethral washings from 20 men who had a needle biopsy of the prostate. In all, 24 patients were found to have prostate cancer and telomerase activity was detected in 14 urine samples. In that study, telomerase activity was detectable in all but one high-grade cancer, with only 40% of low-grade cancers being positive. Another report [37] that assessed epithelial cells obtained after prostatic massage found a sensitivity of 90% and specificity of 76% for telomerase activity in a cohort of 30 patients with prostate cancer and 30 with BPH, using a TRAP assay. The varying results obtained from different groups might be explained by their technique of prostate massage, or might simply represent an issue with the sensitivity of the assay; possibly a significant amount of protein must be released to be detectable.

Teni et al. [38] assessed prostatic inhibin-like peptide, which is involved in the suppression of FSH; they analysed levels in 24-h urine collections and found a sensitivity of 80% and specificity of 100% using 21 patients with cancer and 21 controls. No confirmatory studies have yet been published.

Tissue factor can be expressed by malignant tissue and increased urinary levels were reported in breast and colorectal cancer, and in inflammatory bowel disease. Lwaleed et al. [39] assessed urinary tissue factor in 26 patients with prostate cancer and 57 controls (including women), finding a sensitivity of 65% and specificity of 75%. The urinary tissue factor levels increased with tumour grade. Similar results were reported by Adamson et al. [40], with a sensitivity of 57%, specificity of 75% in 53 cases and 57 controls.

Urinary transferrin levels have also been investigated as potential urinary markers for prostate cancer. Fernandez et al. [41] analysed urine from 22 patients with prostate cancer and compared them with age-matched controls. Transferrin levels were high in 18 of the 22 patients. By contrast, van Diejen et al. [42] examined the diagnostic value of urinary transferrin in 34 men with cancer and 36 controls, finding a sensitivity of 75% and specificity of 30%. They concluded that serum PSA and prostate acid phosphatase level had better diagnostic value.

Urinary PSA has not been as extensively studied as its serum counterpart, but recently there was work undertaken on serum/urine ratios. Graves et al. [43] first reported the presence of PSA in urine in 1985; the evidence to date suggests that the urinary source of PSA is the prostate and the periurethral glands [44]. It was shown by Iwakiri et al. [44]
that PSA is present in the urine of patients after radical prostatectomy, which negates its use as a marker of recurrence. Some was reported on the ratio of serum to urinary PSA, and studies by Irani et al. [45,46] found that this ratio is clinically useful, especially in the 4–10 ng/mL range of PSA (sensitivities of 42–84% and specificity of 80–89%). Other reports confirmed the ability of this ratio to discriminate between BPH and prostate cancer, whilst some others were unable to reproduce these findings [47].

Stoeb et al. [48] used two monoclonal antibodies to detect mini-chromosome maintenance-5 (MCM-5) in urinary sediments in 12 patients with prostate cancer and 201 controls. They used voided urine as their sample and found a sensitivity of 92% and specificity of 82%. They noted MCM-5 was not elevated in patients with BPH. A Japanese group [49] found that a protein-detection method for bradenton (a member of the human septin family) using monoclonal antibody-based immuno-chromatographic membrane strip tests, was able to detect 70% of prostate, renal and bladder cancers in patients’ urine, with no false-positive results. They proposed this as a rapid clinical screening method of urine for cancer. Unfortunately, no information was given about the collection, processing or handling of the urine samples. The report also does not clearly identify the number of urine samples used. A report by Lombardo et al. [50] identified urinary Sreductase type 2 as a potential marker of prostate disease. They identified this using an antibody and found it to be positive in four men with untreated prostate cancer, and negative in the urine of four men on hormonal therapy for prostate cancer. No follow-up study was reported.

An interesting paper by Rehman et al. [51] used gel-based proteomic strategies to identify proteins of interest in urine after prostatic biopsy. They identified calgranulin B-MRP-14 in urine from cancer patients (six) but not in the BPH cohort (six). Although this was a very small sample, the results are potentially very promising. This is the first paper using a proteomic platform to identify a urinary biomarker for prostate cancer.

DISCUSSION

The literature search identified 32 relevant articles for this review; they were subdivided into 11 DNA markers, six RNA markers and 15 protein markers. Unfortunately there were few patients in most of the studies, and there was a wide range in sensitivity and specificity for different markers. DNA- and RNA-based markers rely on cellular shedding into the urine, which is quite different from measuring protein and protein products in the urine, as it requires exfoliation of cells from the tumour as opposed to an alteration in cell metabolism resulting in a change in the protein products of the cell. It is probable that exfoliation of cells into the urine occurs later than an overall change in the proteome of the cell, and hence screening for changes in the proteome might detect cancer earlier than using techniques for cellular detection. In addition, using exfoliated cells for urine analysis presents the problem that well-differentiated, early cancers are less likely to be shed into urine than are poorly differentiated, larger cancers. Thus, a screening test requiring cellular exfoliation (either with or without prostate massage) could potentially miss the early, latent period during which prostate cancer is potentially curable.

Importantly, many patients were recruited for these studies based on a pre-existing suspicion for prostate cancer, due to an elevated serum PSA level or in some cases a suspicious DRE. This would then reflect in a higher sensitivity and specificity than would be expected from a screening programme where patients had not been ‘pre-selected’ as high risk. Some studies used age-matched controls, but it is known that up to 30% of men with a PSA level of <4 ng/mL might harbour foci of prostate cancer [7] and hence this would affect the quoted sensitivity and specificity for the test. Indeed, some authors acknowledge that the sensitivity and specificity of their test would be improved by following their controls to see if they developed prostate cancer [35]. Also, by selecting men at high risk of prostate cancer according to current clinical variables there is the possibility that early markers of prostate cancer might be missed and the urinary molecular markers in the above studies then might reflect a more advanced phenotype, which questions their suitability as de novo screening tools. Most of the studies based histology on prostate biopsy results as opposed to radical prostatectomy specimens (which would give a better indication of the complete histology).

Prostate massage followed by collecting initial voided urine would be expected to increase the ability to detect a given marker of prostatic malignancy; the work by Goessl et al. [16] would certainly appear to support this, but Crocitto et al. [17] found the sensitivity and specificity to be poor in their study, despite prostatic massage. This issue is very important for urinary biomarker discovery, as prostate massage would increase the demands on the physician and probably be poorly tolerated by patients. Also, a protocol for the duration of massage would have to be determined. Thus, the question of whether to massage before collecting the void or to collect a standard urine void can only be addressed in a large-scale clinical trial.

Biomarker discovery programmes using urine samples face many practical issues, e.g. time of collection, spot urine/overnight void vs 24-h collection, the portion of void to be used [52] (having identified that the initial portion of the void contains the highest concentration of PSA, prostatic acid phosphatase and B-inhibin), preserving the stability of the marker (DNA being more stable in urine than RNA) and whether to use prostate massage or not. This aside, urine is readily available and obtainable noninvasively, which makes it ideal for screening.

Most markers identified in this review were protein-based and used ELISA assays to confirm the presence of the postulated biomarkers. This allows diagnosis to proceed ‘one marker at a time’, but the heterogeneity of cancer is such that a panel of markers is a more realistic goal. This would potentially provide information not only on the presence of malignancy but also the likely response to treatment, and the prognosis. Over the last few years the field of proteomics has emerged as a potential tool for developing oncological biomarkers. Proteomic platforms allow for high throughput, and the rapid analysis of hundreds and sometimes thousands of proteins and peptides. By ‘mining’ the urinary proteome the next generation of prostate cancer markers could be identified.

CONCLUSION

It is clear that there are few urinary biomarkers currently under investigation for the detection, monitoring and prediction of progression of prostate cancer. There are limited studies assessing substances known to play a role in prostate cancer, but there are few discovery papers using urine as a starting point and then correlating changes in
expression with occurrence, stage and progression of prostate cancer. Urinary molecular markers might allow early phenotypic cellular changes of malignancy to be identified and hence might complement/ surpass serum biomarkers. Appropriate patient selection is required for studies, e.g. full histology should be available in the form of a radical prostatectomy specimen as opposed to just needle-biopsy material. This will ensure absolute correlation of an identified biomarker with the grade/stage of malignancy and allow prognostic stratification. Optimal sample collection, storage and handling will ensure preservation of potential biomarkers and reduce the variability in what is already by its nature a variable sample type. Finally, proteomic techniques allow for a high-throughput analysis of urine with the visualization and quantification of thousands of potential protein markers [53]. This represents a very promising new tool in the search for new, improved urinary molecular markers of prostate cancer.

CONFLICT OF INTEREST

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REFERENCES

7 Schroder FH, van der Cruysen–Koeter I, de Koning HJ, Vis AN, Hoedemaker RF, Kranse R. Prostate cancer detection at low PSA levels. J Urol 2000; 163: 806–12
8 Christenssen A, Bjork T, Nilsson O et al. Serum prostate specific antigen complexed to eol-1antichymotrypsin as an indicator of prostate cancer. J Urol 1993; 150: 100–5
9 Benson MC, Whang IS, Olsson CA, McMahon DJ, Cooner WH. The use of prostate specific antigen density to enhance the predictive value of intermediate levels of serum PSA. J Urol 1992; 147: 817–21
26 Hessels D, Klein–Gunnemwiek JM, van Oort I et al. DD3 (PCA3)-based molecular urine analysis for the diagnosis of prostate cancer. Eur Urol 2003; 44: 8–16
30 Callakury BV, Brien TP, Lowry CV et al. Telomerase activity in human benign prostatic tissue and prostatic
botchkina glutathione-s-transferase; hgpip, high-grade prostate intraepithelial neoplasia。(q)msp, (quantitative) methylation specific pcr; 8-ohdg, 8-hydroxydeoxyguanosine; rt, reverse transcriptase; amacr, α-methylacyl coenzyme a racemase; trap, telomeric repeat amplification protocol; tb–15, thymosin β15; mcms−5, mini-chromosome maintenance-5.

adrenomedullas. diag mol pathol 1997; 6: 192–8

31 botchkina gi, kim rh, botchkina il, kirshenbaum a, frischer z, adler hl. Noninvasive detection of prostate cancer by quantitative analysis of telomerase activity. clin cancer res 2005; 11: 3243–9

32 hutchinson lm, chang el, becker cm et al. Use of thymosin β15 as a urinary biomarker in human prostate cancer. prostate 2005; 64: 116–27

33 hutchinson lm, chang el, becker cm et al. Development of a sensitive and specific enzyme-linked immunosorbent assay for thymosin β15, a urinary biomarker of human prostate cancer. clin biochem 2005; 38: 558–71

34 bao l, loda m, janney pa, stewart r, anand-apatke b, zetter br. Thymosin β15: a novel regulator of tumour cell motility upregulated in metastatic prostate cancer. nat med 1996; 2: 1322–8

35 rogers cg, yan y, zha s et al. Prostate cancer detection on urinalysis for alpha-methylacyl coenzyme a racemase protein. j urol 2004; 172: 1501–3

36 meid fh, gygi cm, leisinger hj, bosman ft, benhattar j. The use of telomerase activity for the detection of prostate cancer cells after prostatic massage. j urol 2001; 165: 1802–5

37 vicentini c, gravina gl, angelucci a et al. Detection of telomerase activity in prostate massage samples improves differentiating prostate cancer from benign prostatic hyperplasia. j cancer res clin oncol 2004; 130: 217–21


39 lwaleed ba, francis jl, chisholm m. Urinary tissue factor levels in patients with bladder and prostate cancer. eur j surg oncol 2000; 26: 44–9

40 adamson as, francis jl, witherow ro, snell me. Urinary tissue factor levels in prostatic carcinoma: a potential marker of metastatic spread. br j urol 1993; 71: 587–92

41 fernandez c, rifai n, wenger as, mickey dd, silverman lm. A preliminary study of urinary transferrin as a marker for prostate cancer. clin chim acta 1986; 161: 335–9

42 van dieijen-visser mp, hendriks mw, delaere kp, gijzen ah, bромbacher pj. The diagnostic value of urinary transferrin compared to serum prostate specific antigen (psa) and prostate acid phosphatase (pap) in patients with prostate cancer. clin chim acta 1988; 177: 77–80

43 graves hc, sensabaugh gf, blake et. Postcoital detection of a male-specific semen protein. application to the investigation of rape. n engl j med 1985; 312: 338–43

44 iwakiri j, grandbois k, wehner n, graves hc, stamey t. An analysis of urinary prostate specific antigen before and after radical prostatectomy: evidence for secretion of prostate specific antigen by the periurethral glands. j urol 1993; 149: 783–6

45 irani j, salomon l, soulie m et al. Urine/serum prostate-specific antigen ratio: comparison with free/total serum prostate-specific antigen ratio in improving prostate cancer detection. urology 2005; 65: 533–7

46 irani j, millet c, levillian p, dore b, begon f, aubert j. Serum-to-urinary prostate specific antigen ratio: its impact in distinguishing prostate cancer when serum prostate specific antigen level is 4 to 10 ng/ml. j urol 1997; 157: 185–8

47 pannek j, rittenhouse hg, evans cl et al. Molecular forms of prostate-specific antigen and human kallikrein 2 (hk2) in urine are not clinically useful for early detection and staging of prostate cancer. urology 1997; 50: 715–21

48 stoeber k, swinn r, prevost at et al. Diagnosis of genito-urinary tract cancer by detection of minichromosome maintenance 5 protein in urine sediments. j natl cancer inst 2002; 94: 1071–9

49 tanaka m, tanaka t, matsuzaki s et al. Rapid and quantitative detection of human septin family bradeion as a practical diagnostic method of colorectal and urologic cancers. med sci monit 2003; 9: mt61–8

50 lombardo me, hudson pb. Preliminary evaluation of 5α-reductase type 2 in urine as potential marker for prostate disease. steroids 1997; 62: 682–5

51 rehman i, azzouzi ar, catto jw et al. Proteomic analysis of voided urine after prostatic massage from patients with prostate cancer: a pilot study. urology 2004; 64: 1238–43

52 tremblay j, frenette g, tremblay rr, dupont a, thabet m, dube jy. Excretion of three major prostatic secretory proteins in the urine of normal men and patients with benign prostatic hypertrophy or prostate cancer. prostate 1987; 10: 235–43