

The role of circulating tumour cells and nucleic acids in blood for the detection of bladder cancer: a systematic review

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Funding support: No specific funding to disclose

Conflict of interest disclosure: No conflict of interest to disclose

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Abstract

Background

Blood-based biomarkers are a neglected resource in bladder cancer, where the mainstay of focus has been on urinary biomarkers. However, blood-based biomarkers are gaining popularity in other solid cancers, particularly circulating tumour cells (CTCs) and circulating nucleic acids. In this systematic review, we identify and discuss the diagnostic value of CTC, cell-free DNA and RNA based biomarkers in bladder cancer.

Methods

A MEDLINE/Pubmed systematic search was performed using the following keywords: (bladder cancer) AND (blood OR plasma OR serum) AND biomarker AND (DNA OR RNA OR cfDNA OR cell-free DNA OR RNA OR CTC). All studies including blood-based biomarkers based on DNA, RNA and CTCs were reviewed. Of the included studies, studies reporting sensitivity, specificity and/or AUC/ROC values were further described.

Results

Systematic searched yielded 47 studies that were eligible, of which 21, 19 and 3 studies reported DNA, RNA and CTC biomarkers respectively. 15 of these studies included sensitivity, specificity and/or AUC/ROC values. Biomarkers sensitivity and specificity ranged widely at 2.4-97.6% and 43.3-100% respectively. Median number of patients recruited in the studies was 56 (IQR 41-90). Only 3 studies included an independent validation cohort. The highest sensitivity and specificity pairing achieved in the validation cohort was 80.0% and 89.1% respectively.

Conclusions

This systematic review provides a comprehensive overview of the blood-based CTC and nucleic acid biomarkers that have been investigated. An overlap in interest of targets between studies suggests that these could be promising biomarkers, but few biomarkers achieve high sensitivity and specificity, and fewer still have been validated independently.

Word count: 248 words

Highlights

- Sensitivity and specificity values over 90% were reported using different biomarkers, but many were not validated
- Multi-target panels had higher sensitivity than single biomarkers, without compromising specificity
- Only three studies included validation cohorts
- Prospective studies with validation cohorts are required to test the value of diagnostic biomarkers in bladder cancer

Background

Bladder cancer is the ninth most common in the world, with over 430,000 new cases diagnosed in 2012, and 165,000 bladder cancer deaths[1]. In 2014, the cost of bladder cancer care in the USA was estimated to be \$US4.25 billion and had risen over successive years despite the static incidence of the disease[2]. Cystoscopy and CT imaging are the mainstay investigations for the initial diagnosis of bladder cancer[3] and in this setting urinary based biomarkers have been extensively researched[4] and few have received FDA approval. In contrast, there are currently no FDA approved blood-based tests for the detection of metastatic bladder cancer following cystectomy.

Cystectomy is the gold standard radical treatment for invasive bladder cancer. Imaging by CT is recommended as surveillance to detect recurrence as approximately 50% of cases will relapse within 5 years[5]. The recurrence-free survival suggests that occult or micrometastatic disease is present following cystectomy but goes undetected. The resolution of CT imaging is limited and cannot reliably characterise lesions smaller than 1 cm³. Although unproven, it is attractive to postulate that treatment of micrometastatic disease can be most effective when the disease burden is low. In the setting of post cystectomy for curative intent, blood-based biomarkers could provide a means to detect minimal residual disease and possibly before detection by conventional imaging.

Blood-based biomarkers rely on the detection of circulating cancer cells and “cell-free” nucleic acids[6] and utilise new technologies to interrogate genomic and transcriptomic alterations leading to the discovery of promising new biomarkers[7]. This has been relatively successful in cancers including breast and lung cancer [8,9]. The utility of blood-based biomarkers or liquid biopsy can be applied to all bladder cancers, but could be particularly useful post-cystectomy, where urinary biomarkers would not be applicable. The focus of this review will be to evaluate the current evidence for the use of blood-based biomarkers for the detection of bladder cancer.

Introduction

The rationale for the use liquid biopsy is not dissimilar to the many haematology or biochemistry tests clinicians use in daily clinical practice. With the advent of next-generation sequencing and novel circulating cell-capture methods, a blood sample collected in clinic could be analysed for bladder cancer related alterations, having implications on diagnosis, prognosis and therapy selection. The three main substrates discussed in this review are cfDNA, RNA and circulating tumour cells (CTCs).

cfDNA is an attractive substrate for the detection of disease. Cellular DNA is released into the blood following cell death of normal and cancer cells in the form of cfDNA fragments of approximately 150 base pairs[10]. Although cfDNA is present physiologically (plasma: mean 1.8 ng/ml), its levels are increased in many cancer types including (but not limited to) lung[11], ovarian[12], prostate[13], breast[14] and renal carcinoma[15] due to a higher turnover of cells. Large repositories such as TCGA have been harnessed to provide a tailored or personalised approach where sequencing data from circulating genomic substrates could allow for selection of appropriate targeted therapies, or even personalised subsequent monitoring of disease[16]. In lung cancer, cobas[®] EGFR Mutation Test v2 (Roche Molecular Diagnostics) is the first blood-based genomic test[17] with FDA approval. It can accurately identify mutations in the epidermal growth factor receptor (EGFR) using plasma DNA in patients with non-small cell lung cancer (NSCLC) with a sensitivity and specificity of 78-100% and 93-100% respectively[18]. This allows selection of patients for treatment with tyrosine kinase inhibitors used as a second line treatment for metastatic NSCLC[19].

Circulating tumour cells (CTCs) have also shown promise as non-invasive biomarkers. The presence of CTCs in the blood is associated with decreased overall survival in metastatic breast[20], prostate[21] and colorectal[22] cancer patients and represents a prognostic rather than predictive or diagnostic. The CellSearch system (Veridex) has FDA approval for the enumeration of *in-vivo* circulating tumour cells in breast cancer patients[23], and subsequently received FDA approval for use in prostate and colorectal cancers[24]. A limitation of the CellSearch system is the reliance on cancer cells with EpCAM positivity, and as not all metastatic cells express EpCAM, the false negative results are reflected in its negative predictive values[25].

RNA platforms are relatively unexplored in comparison with no FDA approved tests on the market utilising their diagnostic or prognostic potential. However, there is a strong rationale for their use in this field. Messenger RNAs (mRNAs), long non-coding RNAs (lncRNAs) and micro-RNAs (miRNAs) have been explored as potential targets for cancer detection. mRNAs are the direct products of transcription, and can provide real-time information about intracellular. lncRNAs[26] and miRNAs[27] are non-coding RNAs that have been long been regarded as the waste products of transcription, but have been found to be regulators of protein translation.

Using these liquid biopsies, it is possible to assess for tumour heterogeneity[28] and provide an accurate representation of mutational burden. This is different to traditional tissue biopsy, which often sample tissue from a part of the tumour, which can result in only certain sub-clones within a tumour being represented[29]. As liquid biopsies are non-invasively collected, they can also be repeated more frequently than tumour biopsies, and hence can provide real-time information about a patient's disease burden.

In this systematic review, we will discuss all reported DNA, CTC and RNA blood based biomarkers for the detection of bladder cancer.

Methods

Search strategy and included studies

A systemic review of the literature was performed using Medline/Pubmed on 22nd February 2017. The following keywords/ MeSH words: (bladder cancer) AND (blood OR plasma OR serum) AND biomarker AND (DNA OR RNA OR cfDNA OR cell-free DNA OR RNA OR CTC). All articles were reviewed in accordance with the PRISMA statement. The review is registered with the PROSPERO register (CRD42016051201).

Study selection

All studies were screened by two investigators independently. Where there were disagreements, this was resolved after discussion with a third investigator by general consensus. The inclusion criteria includes: 1) blood-based (blood/plasma/serum) genomic (DNA/RNA) biomarkers or biomarker plans for bladder cancer, 2) diagnostic biomarkers. An in-depth analysis of sensitivity, specificity and/or AUC reporting was conducted for studies reporting relevant data. Only studies in English were included.

All conference abstracts, review articles, editorials, comments, letters to the editor and duplicate records were excluded. Studies on prognostic biomarkers, urinary biomarkers and non-genomic/non-CTC biomarkers were also excluded from analysis. The selection process is summarised in Figure 1.

Data extraction and quality assessment

Data was extracted independently by two investigators (PK, MWLL) from suitable studies about type of biomarker used, region/substrate identified and blood product interrogated. For studies describing blood and urine biomarkers, only blood-based biomarkers were included.

All suitable manuscripts describing blood-based biomarkers were then reviewed for reported statistics: sensitivity, specificity and ROC/AUC values. Additional data was collected for studies that included validation cohorts. A second investigator confirmed data was extracted accurately.

Results

Characterisation of studies

The PRISMA flowchart is shown in Figure 1. A total of 275 citations were identified in the database search, and 47 studies met the criteria of diagnostic biomarkers. The various methods described interrogated CTCs, DNA (both somatic and epigenetic alterations), RNA (miRNA, total RNA, cell-free RNA) and combinations of these strategies. A summary of the included studies is shown in Supplementary Table 1.

21 studies described DNA-based tests, of which 11 described somatic mutation analysis, nine use DNA methylation based analysis and one mitochondrial DNA. Of the 19 RNA studies, 11 studies were based on detection of miRNA, one cellular RNA and seven mRNA. Three studies described CTC analysis, and four used combined approaches of these methods.

Of all studies included in the search, only 15 provided sensitivity and specificity analysis, and/or AUC from ROC analysis. These studies were included for further discussion; six were miRNA based, two studies investigated mRNA and six focused on cfDNA. Of these six studies, three focused on cfDNA somatic mutations and the remaining on cfDNA methylation changes. Generally, these were small studies with a median of 56 (IQR 41-90) patients recruited. There was variability in reporting matched controls and validation sets. A summary of findings of these studies is shown in Table 1.

These 14 studies were assessed against the Standards for Reporting Diagnostic accuracy studies (STARD) criteria. In general, all studies reported majority of the 30 items described in the STARD statement, but some items were missing as a trend across various studies. This includes reporting if patients formed a consecutive, random or convenience series, rationale for test positivity cut-off, determination of sample size, and comparison of the distribution of the index test results to the reference standard.

RNA biomarkers

In the field of bladder cancer, three types of RNA biomarkers have been reported: miRNA, cfRNA and total RNA. miRNAs are small (18-25 nucleotides) non-coding RNA molecules that are involved in a wide range of biological processes, influencing gene regulation and signal transduction in cell development, proliferation and redirecting or reprogramming molecular pathways[27]. mRNAs are direct products of DNA transcription, which code for specific amino acids and act as blueprints for protein synthesis. Therefore, they can be considered as a real-time surrogate of cellular protein synthesis. Total RNA isolated from blood consist mostly of blood-cell derived RNA, and previous studies have hypothesised that blood-derived total RNAs can distinguish between gastric cancer and healthy controls[30]. Additionally, the use of containers such as the PAXgene™ Blood RNA System allows for easier transport and storage of RNA, further enabling researchers to explore the potential of RNA-based biomarkers[31].

miRNA biomarkers

Yang *et al.*[32] studied serum levels of miR-210 which is upregulated in most solid tumours including bladder cancer, but is not cancer specific and is also upregulated in hypoxic conditions[33]. A sensitivity for detection of bladder cancer of 97.6%, and specificity of 69.2% with an AUC of 0.898 was reported in a development cohort, but the findings were not validated using an independent cohort. Tölle *et al.*[34] described three miRNAs (miR-26b-5p, miR-144 5p, miR-374-5p) of which miR-26b-5p and miR-374-5p

had sensitivity of 65.0% and 60.0% respectively with a combined specificity of 94.1% for the detection of invasive cancer. The combined sensitivities for the three miRNAs were not reported and the results were not validated in an independent cohort.

A comprehensive study including a panel of 6 miRNAs was reported by Jiang *et al.*, which included both discovery and validation cohorts[35]. In the development cohort of 240 patients (120 BCs and 120 normal controls), a pooled sensitivity and specificity of 90.0% and 90.0% respectively was reported, and an AUC of 0.956 (0.922–0.978). AUC for the six miRNAs ranged between 0.645 and 0.814, but sensitivity and specificity for individual miRNAs were not included. Independent cohort validation included 110 BCs and 110 normal controls and achieved a sensitivity and specificity of 80.0% and 89.1% respectively, with an AUC of 0.899 (0.851–0.936). Higher detectable levels of miR-152 and lower levels of miR-3187-3p correlated with advanced tumour stage ($p<0.05$), and lower levels of miR-27a-3p correlated with higher tumour grade ($p=0.04$).

Adam *et al.* studied a panel of 40 miRNAs[36] in plasma and reported pooled sensitivity and specificity of 90% and 89% respectively. However, the patient cohort studied consisted of only 20 BCs and 18 non-cancer controls, and no validation cohort was included.

cfRNA biomarkers

A single study has reported the utility of cell-free mRNA isolated from serum as a biomarker for detection of bladder cancer. Ismail *et al.*[37] reported results from 120 bladder cancer patients, stages T1 ($n=30$) and $\geq T2$ ($n=90$), and normal controls ($n=30$)[37]. In this study, mRNA from 6 different S100A genes– S100A4, S100A6, S100A7, S100A8, S100A9 and S100A11 were included. The S100 genes transcribe for calcium-binding proteins which are implicated in cellular processes such as transcription, cell proliferation and differentiation, and expression is associated with bladder cancer[38]. Optimal sensitivity and specificity was achieved with S100A4 at 90% and 92% respectively, and a PPV and NPV of 88.5% and 93.0% respectively. S100A4 was also overexpressed in invasive bladder tumours compared to non-muscle invasive tumours. When combined with urine cytology, the sensitivity increased to 96.6% but the specificity was reduced to 80.0%.

Total RNA biomarkers

Osman[39] *et al.* studied total RNA for a panel of seven primers corresponding to seven genes isolated from nucleated cells in the blood. The approach, to extract RNA from nucleated cells, is different from all other studies reported in this review, which have identified targets in cell-free genomic material. The seven genes (*IGFBP7*, *SNX16*, *CSPG6*, *CTSD*, *CHD2*, *NELL2*, *TNFRSF7*) panel achieved a sensitivity of 83.0%, a specificity of 93.0% and an AUC of 0.901 using a cohort of 44 bladder cancer patients and 29 normal controls[39].

cfDNA biomarkers

Cancer-related DNA changes can be in the form of somatic mutations and epigenetic alterations. Somatic mutations are changes in the DNA sequence itself, whereas epigenetic alterations are the result of methyl groups being added (methylation) to intact DNA molecules, resulting in a change of activity of the DNA segment without changing its sequence.

Ellinger *et al.* reported that 124 base-pair apoptotic DNA fragment of the *PTGS2* (*COX2*) gene has a sensitivity of 95.6%, and specificity of 62.2% and an AUC of 0.836 (0.753–0.918) for detection of bladder cancer. The NPV and PPV of 70.7% and 96.6% respectively was reported for a mixed cohort of MIBC and

NMIBC[40]. DNA levels and apoptotic index were not correlated with stage or grade. In a further study, Ellinger and colleagues detected methylation of 5 loci from the genes *APC*, *GSTP1* or *TIG1* as a biomarker panel for detection of disease. The combination achieved a sensitivity and specificity of 80.0% and 93.3% respectively, and an AUC of 0.867 (0.785–0.948). Individually, all three biomarkers had a specificity of 100% but sensitivity and AUC were lower, ranging between 0.659 and 0.798. Interestingly, they found that adding *PTGS2* to the panel did not affect the sensitivity, specificity and AUC.

Another study by Hauser *et al.* compared the relative ratio of two amplicons from the beta actin gene, a 106pb and a 384pb region, which represents cell free apoptotic DNA compared to large fragment cellular DNA respectively. They reported a sensitivity and specificity of 91.6% and 43.3% interrogating *ACTB* and an AUC of 0.686[41]. Absolute levels of cfDNA and DNA integrity were similar in both NMIBC and MIBC patient samples. Interestingly, the authors infer that increased levels of cfDNA were detected post-surgical resection indicating the potential dynamic release of DNA fragments by processes such as necrosis and repair.

The highest specificity from any of the studies included was reported for p16^{INK4a} promoter methylation. p16^{INK4a} inhibits the activities of CDK4 and is frequently inactivated in cancer[42]. Valenzuela *et al.* studied hypermethylation of the gene as a surrogate for its inactivation in bladder cancer and reported specificity of 98% but sensitivity of only 23%. AUC was not reported but their positive predictive (PPV) was 95%[43].

CTC biomarkers

Only one study has enumerated CTCs. Qi *et al.* analysed a cohort of 20 bladder cancer patients and 23 healthy volunteers, they reported a sensitivity and specificity of 82.1% and 61.9% respectively[44], with an ROC of 0.819 (0.738-0.883), using the CTC unit (Cu) developed by GenoSaber Biotech (Shanghai, China). Their publication included patients with NMIBC, MIBC and metastatic bladder cancer, but performance of their test for individual cohorts was not stated.

Discussion

In the field of cancer diagnostics, interest in the use of blood-based genomic biomarkers has grown in recent years[45,46]. In bladder cancer, blood-based biomarkers may have greatest potential for the detection of residual metastatic disease post radical cystectomy. However, this review found preliminary reports where blood-based biomarkers have been applied for the diagnosis of bladder cancer of all stages.

As evident in this review, research in this field is at an early stage, and most studies are proof-of-concept with limitations in design and cohort size. Only 14 reports were eligible for inclusion, of which no single publication fulfilled the standard specified by the STARD criteria. There was heterogeneity in patient cohorts included in each publication, with variable inclusions of NMIBC and MIBC and stratification by grading. Only one study included patients with metastatic bladder cancer. Furthermore, as all studies included in this review are case-control studies, and use optimal AUC values, there is an increased risk of bias – as is to be expected for early stage biomarker discovery.

Across the review, diverse substrates and platforms are reported, however an emerging focus is the investigation of miRNAs and targets in cfDNA over circulating tumour cells and the presence of mRNA. There is some commonality of targets, such as *PTGS2*, *APC*, *TIG1*, *GSTP1* and *p16* genes which were tested across multiple studies interrogating cfDNA as single targets or as part of biomarker panels for the detection of bladder cancer. *PTGS2* was included in both somatic mutation panels and methylation targets, whereas *APC*, *TIG1*, *GSTP1* and *p16* were included as part of methylation targets associated with bladder cancer. Many targets such as hypermethylation of the *PTGS2* are not cancer specific, but upregulated in inflammation[47], and has been associated with colorectal cancer[48], benign prostatic hyperplasia[49] and osteoarthritis[50] among other conditions. Similarly APC mutations are associated with ovarian, colorectal and hepatocellular[51–53] carcinoma and TIG1, GSTP1 and p16 gene mutations and methylation changes are not individually specific to bladder cancer. This has made their use as part of a panel more appealing, as their sensitivity and specificity for bladder cancer can be pooled and aggregated.

miRNA targets are attractive biomarker targets as they are stable enough to be well preserved and extracted from fixed tissue, blood and urine. Unlike the trend noted in cfDNA, the only miRNA that was used by more than one study was miR-148b, and it was part of both multi-miRNA panels designed by Jiang *et al.*[35] and Adam *et al.*[36]. miR-148b is not specific to bladder cancer, and has even been investigated as a marker for heart failure[54] which itself is not uncommon in the population with bladder cancer[55]. However, the overlap between cancers or other conditions does not exclude the test from monitoring for disease recurrence. When used as part of biomarker panels, they can be interpreted in relation to the presence of other bladder-cancer associated alterations.

Five studies in our literature search described CTCs, and only one met our criteria for further analysis. CTCs have a key area of interest in the diagnosis of many cancers, including bladder cancer, but can be a cost-prohibitive approach[56]. Using the CellSearch platform, CTCs are detectable in 26.1-91% of patients with lymph node metastases[57,58], but also detectable in patients without lymph node metastases. In patients with systemic distant metastases, CTCs are detectable in 33-100% of patients, with higher numbers of CTCs enumerated compared to non-metastatic disease[59]. As such, CTCs can offer prognostic information[60], but do not provide the sensitivity or specificity needed for diagnostic certainty.

In five studies, the type of cancer (urothelial cell carcinoma (UCC), adenocarcinoma, squamous cell carcinoma (SCC) etc.) was not stated, and in eight studies four included UCC only while four included UCC and SCC. There was no consistency in the design of controls and control cohort comprised blood derivatives from variable sources such as: patients with a prior history of bladder cancer[61], patients with known prostate cancer[62], as well as non-cancer controls [36][37,63] without age and sex matching. Of the 14 studies included, only 3 studies (Table 2) validated their findings with an independent cohort, and of these, only 2 studies published by Jiang *et al.*[35] and Du *et al.*[64] included the results obtained from the validation cohort. It is difficult to draw meaningful conclusions about studies without results from a validation cohort, especially given the small number of patients in many of these studies. Interestingly, all three studies that included validation cohorts were interrogating miRNA or miRNA panels as a diagnostic biomarker.

Serum was favoured over plasma by most studies, with 10 studies collecting genomic material from serum, and 2 from plasma, 1 whole blood. Previous studies have found that serum has a higher concentration of miRNA and cfDNA when compared with plasma[65]. However, serum is comparatively fragile and requires immediate separation[66]. Studies included in this review that extracted genomic material from plasma used standard EDTA tubes for blood collection, but blood can be stored using novel storage containers such as Cell-Free DNA BCT®[67] for up to a week.

Another important application of a biomarker is to differentiate patient disease by stage and grade of bladder cancer. Yang *et al.* identified that miR-210 could have a role in differentiating NMIBC patients from MIBC, with an AUC of (95% CI, 0.662–0.800). Furthermore, miR-210 could differentiate MIBC from normal with an AUC of 0.938 (95% CI, 0.893–0.968), compared with NMIBC from control with an AUC of 0.858 (95% CI, 0.800–0.904). This suggests that likelihood of detection is related with disease burden, and similar results were found by other studies[36,37,40,68]. Fewer studies reported correlations of disease detection being related to cancer grades[35,68,69], but limited sample sizes and lack of validation limit the impact of these findings.

The field of genomic biomarkers is relatively more developed in other cancers. In breast cancer, a cfDNA based biomarker could detect cancer recurrence approximately eight months before conventional scans[46]. Using a personalised cfDNA panel, the TRACERx group detected disease recurrence in lung cancer approximately 2 months before conventional scans[8]. It must be noted that these tests are not recommended for clinical use. In bladder cancer, there are no reported studies reporting prospective sequential sampling. However, the reports identified in this review set out that blood-based biomarkers can be detected upon clinical diagnosis and prior to resection, although with varying sensitivities and specificities. Future studies exploring the potential for blood-based biomarkers to supplement cross-sectional imaging will be of interest.

Although not tested as a readout of minimal residual disease, the finding that circulating genomic material is rapidly cleared makes it attractive as a biomarker. In nasopharyngeal carcinoma, cancer-associated Epstein-Barr Virus DNA becomes undetectable in a few days following surgical resection suggesting that the half-life of genomic material in the circulation is around 2 hours[70] and can be as short as 16 minutes[71]. Sensitivities as high as 95% have been reported for the detection of PTGS2 in bladder cancer[62], suggesting some utility as a readout of minimal residual disease if detected after cystectomy.

Liquid biopsies can have inference for the monitoring or disease status and response to therapy. In our literature search, non-diagnostic biomarkers were also identified but excluded for systematic analysis. These included biomarkers predictive of metastatic disease, chemo-sensitivity, recurrence in NMIBC and prognosis. While not included in the scope of this systematic review, they represent important applications of genomic biomarkers.

Circulating nucleic acids and CTCs have the potential to be useful biomarkers for bladder cancer. Small exploratory studies in the literature have shown promising results for blood-based biomarkers in the diagnosis of bladder cancer, but these need to be robustly tested and validated for them to have a role in the clinical setting. The implication that evidence of tumour burden can be detected through non-invasive methods is an important avenue to explore further.

Acknowledgements

We are grateful to the Medical Research Council (JDK, AF), Urology Foundation (WST, PK), Mason Medical Research Foundation (WST) & UCLH Biomedical Research Centre (JDK) for funding our work.

References

- [1] Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. *Eur Urol* 2017;71:96–108. doi:10.1016/j.eururo.2016.06.010.
- [2] Yeung C, Dinh T, Lee J. The Health Economics of Bladder Cancer: An Updated Review of the Published Literature. *Pharmacoeconomics* 2014;32:1093–104. doi:10.1007/s40273-014-0194-2.
- [3] Witjes JA, Compérat E, Cowan NC, De Santis M, Gakis G, Lebreton T, et al. EAU Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2013 Guidelines. *Eur Urol* 2014;65:778–92. doi:10.1016/j.eururo.2013.11.046.
- [4] Herman MP, Svatek RS, Lotan Y, Karakiewicz PI, Shariat SF. Urine-based biomarkers for the early detection and surveillance of non-muscle invasive bladder cancer. *Minerva Urol Nefrol* 2008;60:217–35.
- [5] Ghoneim MA, Abdel-Latif M, El-Mekresh M, Abol-Enein H, Mosbah A, Ashamalla A, et al. Radical cystectomy for carcinoma of the bladder: 2,720 consecutive cases 5 years later. *J Urol* 2008;180:121–7. doi:10.1016/j.juro.2008.03.024.
- [6] Uttley L, Whiteman BL, Woods HB, Harnan S, Philips ST, Cree IA. Building the Evidence Base of Blood-Based Biomarkers for Early Detection of Cancer: A Rapid Systematic Mapping Review. *EBioMedicine* 2016;10:164–73. doi:10.1016/j.ebiom.2016.07.004.
- [7] Feber A, Dhama P, Dong L, de Winter P, Tan WS, Martínez-Fernández M, Paul DS, Hynes-Allen A, Rezaee S, Gurung P, Rodney S, Mehmood A, Villacampa F, de la Rosa F, Jameson C, Cheng KK, Zeegers MP, Bryan RT, James ND, Paramio JM, Freeman A, Beck S KJ. UroMark - A urinary biomarker assay for the detection of bladder cancer. *Clin Epigenetics* 2016.
- [8] Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. *Nature* 2017;545:446–51. doi:10.1038/nature22364.
- [9] Garcia-Murillas I, Schiavon G, Weigelt B, Ng C, Hrebien S, Cutts RJ, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer. *Sci Transl Med* 2015;7:302ra133. doi:10.1126/scitranslmed.aab0021.
- [10] Volik S, Alcaide M, Morin RD, Collins CC. Cell-free DNA (cfDNA): clinical significance and utility in cancer shaped by emerging technologies. *Mol Cancer Res* 2016;14:molcanres.0044.2016. doi:10.1158/1541-7786.MCR-16-0044.
- [11] Gautschi O, Bigosch C, Huegli B, Jermann M, Marx A, Chassé E, et al. Circulating deoxyribonucleic Acid as prognostic marker in non-small-cell lung cancer patients undergoing chemotherapy. *J Clin Oncol* 2004;22:4157–64. doi:10.1200/JCO.2004.11.123.
- [12] Chang H-W, Lee SM, Goodman SN, Singer G, Cho SKR, Sokoll LJ, et al. Assessment of plasma DNA levels, allelic imbalance, and CA 125 as diagnostic tests for cancer. *J Natl Cancer Inst* 2002;94:1697–703.
- [13] Ellinger J, Haan K, Heukamp LC, Kahl P, Büttner R, Müller SC, et al. CpG island hypermethylation in cell-free serum DNA identifies patients with localized prostate cancer. *Prostate* 2008;68:42–9. doi:10.1002/pros.20651.
- [14] Pölcher M, Ellinger J, Willems S, El-Maarri O, Höller T, Amann C, et al. Impact of the menstrual cycle on circulating cell-free DNA. *Anticancer Res* 2010;30:2235–40.
- [15] de Martino M, Klatté T, Haitel A, Marberger M. Serum cell-free DNA in renal cell carcinoma: a diagnostic and prognostic marker. *Cancer* 2012;118:82–90. doi:10.1002/cncr.26254.
- [16] Carpinetti P, Donnard E, Bettoni F, Asprino P, Koyama F, Rozanski A, et al. The use of personalized biomarkers and liquid biopsies to monitor treatment response and disease recurrence in locally advanced rectal cancer after neoadjuvant chemoradiation. *Oncotarget* 2015;6:38360–71. doi:10.18632/oncotarget.5256.
- [17] U.S. Food & Drug Administration. cobas EGFR Mutation Test v2 2016. <http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm504540.htm> (accessed November 3, 2016).
- [18] Thress KS, Brant R, Carr TH, Dearden S, Jenkins S, Brown H, et al. EGFR mutation detection in ctDNA from NSCLC patient plasma: A cross-platform comparison of leading technologies to support the clinical development of AZD9291. *Lung Cancer* 2015;90:509–15. doi:10.1016/j.lungcan.2015.10.004.

- [19] Novello S, Barlesi F, Califano R, Cufer T, Ekman S, Giaj Levra M, et al. Metastatic nonsmallcell lung cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and followup 2016;27. doi:10.1093/annonc/mdw326.
- [20] Bidard F-C, Peeters DJ, Fehm T, Nolé F, Gisbert-Criado R, Mavroudis D, et al. Clinical validity of circulating tumour cells in patients with metastatic breast cancer: a pooled analysis of individual patient data. *Lancet Oncol* 2014;15:406–14. doi:10.1016/S1470-2045(14)70069-5.
- [21] de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, et al. Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. *Clin Cancer Res* 2008;14:6302–9. doi:10.1158/1078-0432.CCR-08-0872.
- [22] Cohen SJ, Punt CJA, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, et al. Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol* 2008;26:3213–21. doi:10.1200/JCO.2007.15.8923.
- [23] U.S. Food & Drug Administration. Medical Procedures Device Approvals and Clearances Recently-Approved Devices CellSearch™ Epithelial Cell Kit / CellSpotter™ Analyzer - K031588 n.d. <http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/Recently-ApprovedDevices/ucm081239.htm> (accessed November 3, 2016).
- [24] Office of In Vitro Diagnostic Device Evaluation and Safety. Summary Information (510(k) safety and effectiveness information 2010. https://www.accessdata.fda.gov/cdrh_docs/pdf10/k103502.pdf (accessed February 14, 2018).
- [25] Thurm H, Ebel S, Kentenich C, Hensen A, Riethdorf S, Coith C, et al. Rare expression of epithelial cell adhesion molecule on residual micrometastatic breast cancer cells after adjuvant chemotherapy. *Clin Cancer Res* 2003;9:2598–604.
- [26] Kohls K, Schmidt D, Holdenrieder S, Müller SC, Ellinger J. [Detection of cell-free lncRNA in serum of cancer patients]. *Urologe A* 2015;54:819–25. doi:10.1007/s00120-014-3655-5.
- [27] Moro-Soria A. MicroRNAs as biomarkers and therapeutic targets in cancer. *Biotechnol Apl* 2014;31:87–92. doi:10.1016/j.trsl.2011.01.013.
- [28] Burrell RA, McGranahan N, Bartek J, Swanton C. The causes and consequences of genetic heterogeneity in cancer evolution. *Nature* 2013;501:338–45. doi:10.1038/nature12625.
- [29] De Mattos-Arruda L, Weigelt B, Cortes J, Won HH, Ng CKY, Nuciforo P, et al. Capturing intra-tumor genetic heterogeneity by de novo mutation profiling of circulating cell-free tumor DNA: A proof-of-principle. *Ann Oncol* 2014;25:1729–35. doi:10.1093/annonc/mdu239.
- [30] Chen S, Zhu J, Yu F, Tian Y, Ma S, Liu X. Combination of miRNA and RNA functions as potential biomarkers for gastric cancer. *Tumor Biol* 2015;36:9909–18. doi:10.1007/s13277-015-3756-9.
- [31] Rainen L, Oelmueller U, Jurgensen S, Wyrich R, Ballas C, Schram J, et al. Stabilization of mRNA expression in whole blood samples. *Clin Chem* 2002;48:1883–90.
- [32] Yang Y, Qu A, Liu J, Wang R, Liu Y, Li G, et al. Serum miR-210 contributes to tumor detection, stage prediction and dynamic surveillance in patients with bladder cancer. *PLoS One* 2015;10:1–12. doi:10.1371/journal.pone.0135168.
- [33] Siwaponanan P, Fucharoen S, Sirankapracha P, Winichagoon P, Umemura T, Svasti S. Elevated levels of miR-210 correlate with anemia in β -thalassemia/HbE patients. *Int J Hematol* 2016;104:338–43. doi:10.1007/s12185-016-2032-0.
- [34] Tölle A, Jung M, Rabenhorst S, Kilic E, Jung K, Weikert S. Identification of microRNAs in blood and urine as tumour markers for the detection of urinary bladder cancer. *Oncol Rep* 2013;30:1949–56. doi:10.3892/or.2013.2621.
- [35] Jiang X, Du L, Wang L, Li J, Liu Y, Zheng G, et al. Serum microRNA expression signatures identified from genome-wide microRNA profiling serve as novel noninvasive biomarkers for diagnosis and recurrence of bladder cancer. *Int J Cancer* 2015;136:854–62. doi:10.1002/ijc.29041.
- [36] Adam L, Wszolek MF, Liu CG, Jing W, Diao L, Zien A, et al. Plasma microRNA profiles for bladder cancer detection. *Urol Oncol Semin Orig Investig* 2013;31:1701–8. doi:10.1016/j.urolonc.2012.06.010.

- [37] Ismail MF, El Boghdady NA, Shabayek MI, Awida HA, Abozeed H. Evaluation and screening of mRNA S100A genes as serological biomarkers in different stages of bladder cancer in Egypt. *Tumor Biol* 2016;37:4621–31. doi:10.1007/s13277-015-4264-7.
- [38] Shiota M, Tsunoda T, Song Y, Yokomizo A, Tada Y, Oda Y, et al. Enhanced S100 calcium-binding protein P expression sensitizes human bladder cancer cells to cisplatin. *BJU Int* 2011;107:1148–53. doi:10.1111/j.1464-410X.2010.09535.x.
- [39] Osman I, Bajorin DF, Sun T-T, Zhong H, Douglas D, Scattergood J, et al. Novel Blood Biomarkers of Human Urinary Bladder Cancer. *Clin Cancer Res* 2006;12:3374–80. doi:10.1158/1078-0432.CCR-05-2081.
- [40] Ellinger J, Bastian PJ, Ellinger N, Kahl P, Perabo FG, Büttner R, et al. Apoptotic DNA fragments in serum of patients with muscle invasive bladder cancer: A prognostic entity. *Cancer Lett* 2008;264:274–80. doi:10.1016/j.canlet.2008.01.038.
- [41] Hauser S, Kogej M, Fechner G, Von Ruecker A, Bastian PJ, Von Pezold J, et al. Cell-free serum DNA in patients with bladder cancer: results of a prospective multicenter study. *Anticancer Res* 2012;32:3119–24.
- [42] Serrano M. The Tumor Suppressor Protein p16 INK4a. *Exp Cell Res* 1997;13:7–13. doi:10.1006/excr.1997.3824.
- [43] Valenzuela MT, Galisteo R, Zuluaga A, Villalobos M, Nunez MI, Oliver FJ, et al. Assessing the use of p16(INK4a) promoter gene methylation in serum for detection of bladder cancer. *Eur Urol* 2002;42:622–30.
- [44] Qi F, Liu Y, Zhao R, Zou X, Zhang L, Li J, et al. Quantitation of rare circulating tumor cells by folate receptor α ligand-targeted PCR in bladder transitional cell carcinoma and its potential diagnostic significance. *Tumor Biol* 2014;35:7217–23. doi:10.1007/s13277-014-1894-0.
- [45] Forshew T, Murtaza M, Parkinson C, Gale D, Tsui DWY, Kaper F, et al. Noninvasive identification and monitoring of cancer mutations by targeted deep sequencing of plasma DNA. *Sci Transl Med* 2012;4:136ra68–136ra68. doi:10.1126/scitranslmed.3003726.
- [46] Garcia-murillas I, Schiavon G, Weigelt B, Ng C, Hrebien S, Cutts RJ, et al. Mutation tracking in circulating tumor DNA predicts relapse in early breast cancer 2015;7:1–12. doi:10.1126/scitranslmed.aab0021.
- [47] Moon CM, Kwon J-H, Kim JS, Oh S-H, Jin Lee K, Park JJ, et al. Nonsteroidal anti-inflammatory drugs suppress cancer stem cells via inhibiting PTGS2 (cyclooxygenase 2) and NOTCH/HES1 and activating PPAR γ in colorectal cancer. *Int J Cancer* 2014;134:519–29. doi:10.1002/ijc.28381.
- [48] Vogel LK, Sæbø M, Høyer H, Kopp TI, Vogel U, Godiksen S, et al. Intestinal PTGS2 mRNA levels, PTGS2 gene polymorphisms, and colorectal carcinogenesis. *PLoS One* 2014;9:e105254. doi:10.1371/journal.pone.0105254.
- [49] St Sauver JL, Lieber MM, Slager SL, Jacobson DJ, McGree ME, Jacobsen SJ. Associations between variants in the cyclooxygenase 2 enzyme gene (PTGS2) and development of benign prostate enlargement. *BJU Int* 2011;108:1610–5. doi:10.1111/j.1464-410X.2011.10161.x.
- [50] Güler VG, Yalın S, Berköz M, Cimen OB, Yalın E, Eroğlu P, et al. The association between cyclooxygenase-2 (COX-2/PTGS2) gene polymorphism and osteoarthritis. *Eklemler Hastalıkları Cerrahisi* 2011;22:22–7.
- [51] Chen T-H, Chang S-W, Huang C-C, Wang K-L, Yeh K-T, Liu C-N, et al. The prognostic significance of APC gene mutation and miR-21 expression in advanced-stage colorectal cancer. *Colorectal Dis* 2013;15:1367–74. doi:10.1111/codi.12318.
- [52] Bhagat R, Chadaga S, Premalata CS, Ramesh G, Ramesh C, Pallavi VR, et al. Aberrant promoter methylation of the RASSF1A and APC genes in epithelial ovarian carcinoma development. *Cell Oncol (Dordr)* 2012;35:473–9. doi:10.1007/s13402-012-0106-4.
- [53] Jain S, Chang T-T, Hamilton JP, Lin SY, Lin Y-J, Evans AA, et al. Methylation of the CpG sites only on the sense strand of the APC gene is specific for hepatocellular carcinoma. *PLoS One* 2011;6:e26799. doi:10.1371/journal.pone.0026799.
- [54] Chen MC, Chang TH, Chang JP, Huang H Da, Ho WC, Lin YS, et al. Circulating miR-148b-3p and miR-409-3p as biomarkers for heart failure in patients with mitral regurgitation. *Int J Cardiol* 2016;222:148–54. doi:10.1016/j.ijcard.2016.07.179.
- [55] American Cancer Society. Key statistics for bladder cancer n.d. <http://www.cancer.org/cancer/bladdercancer/detailedguide/bladder-cancer-key-statistics> (accessed November 3, 2016).

- [56] Liu Z, Guo W, Zhang D, Pang Y, Shi J, Wan S, et al. Circulating tumor cell detection in hepatocellular carcinoma based on karyoplasmic ratios using imaging flow cytometry. *Sci Rep* 2016;6:39808. doi:10.1038/srep39808.
- [57] Rink M, Chun FK, Dahlem R, Soave A, Minner S, Hansen J, et al. Prognostic role and HER2 expression of circulating tumor cells in peripheral blood of patients prior to radical cystectomy: A prospective study. *Eur Urol* 2012;61:810–7. doi:10.1016/j.eururo.2012.01.017.
- [58] Okegawa T, Hayashi K, Hara H, Nutahara K, Higashihara E. Immunomagnetic quantification of circulating tumor cells in patients with urothelial cancer. *Int J Urol* 2010;17:254–8. doi:10.1111/j.1442-2042.2010.02454.x.
- [59] Soave A, Riethdorf S, Pantel K, Fisch M, Rink M. Do Circulating Tumor Cells Have a Role in Deciding on Adjuvant Chemotherapy After Radical Cystectomy? *Curr Urol Rep* 2015;16:46. doi:10.1007/s11934-015-0520-z.
- [60] Gazzaniga P, De Berardinis E, Raimondi C, Gradilone A, Busetto GM, De Falco E, et al. Circulating tumor cells detection has independent prognostic impact in high-risk non-muscle invasive bladder cancer. *Int J Cancer* 2014;135:1978–82. doi:10.1002/ijc.28830.
- [61] Hauser S, Kogej M, Fechner G, Von Pezold J, Vorreuther R, Lummen G, et al. Serum DNA hypermethylation in patients with bladder cancer: Results of a prospective multicenter study. *Anticancer Res* 2013;33:779–84.
- [62] Ellinger J, El Kassem N, Heukamp LC, Matthews S, Cubukluoz F, Kahl P, et al. Hypermethylation of Cell-Free Serum DNA Indicates Worse Outcome in Patients With Bladder Cancer. *J Urol* 2008;179:346–52. doi:10.1016/j.juro.2007.08.091.
- [63] von Knobloch R, Hegele a, Brandt H, Olbert P, Heidenreich a, Hofmann R. Serum DNA and urine DNA alterations of urinary transitional cell bladder carcinoma detected by fluorescent microsatellite analysis. *Int J Cancer* 2001;94:67–72. doi:10.1002/ijc.1434.
- [64] Du M, Shi D, Yuan L, Li P, Chu H, Qin C, et al. Circulating miR-497 and miR-663b in plasma are potential novel biomarkers for bladder cancer. *Sci Rep* 2015;5:10437. doi:10.1038/srep10437.
- [65] Lee TH, Montalvo L, Chrebtow V, Busch MP. Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma. *Transfusion* 2001;41:276–82.
- [66] Tuck MK, Chan DW, Chia D, Godwin AK, Grizzle WE, Krueger KE, et al. Standard Operating Procedures for Serum and Plasma Collection. *J Proteome Res* 2010;8:113–7. doi:10.1021/pr800545q.Standard.
- [67] Cell-Free DNA BCT® - Streck n.d. <https://www.streck.com/product.aspx?p=cell-free dna bct> (accessed November 4, 2016).
- [68] Von Knobloch R, Brandt H, Schrader AJ, Heidenreich A, Hofmann R. Molecular Serological Detection of DNA Alterations in Transitional Cell Carcinoma Is Highly Sensitive and Stage Independent. *Clin Cancer Res* 2004;10:988–93. doi:10.1158/1078-0432.CCR-0851-3.
- [69] Scheffer AR, Holdenrieder S, Kristiansen G, von Ruecker A, Müller SC, Ellinger J. Circulating microRNAs in serum: Novel biomarkers for patients with bladder cancer? *World J Urol* 2014;32:353–8. doi:10.1007/s00345-012-1010-2.
- [70] To EWH, Chan KCA, Leung S-F, Chan LYS, To K-F, Chan ATC, et al. Rapid clearance of plasma Epstein-Barr virus DNA after surgical treatment of nasopharyngeal carcinoma. *Clin Cancer Res* 2003;9:3254–9.
- [71] Lo YM, Zhang J, Leung TN, Lau TK, Chang AM, Hjelm NM. Rapid clearance of fetal DNA from maternal plasma. *Am J Hum Genet* 1999;64:218–24. doi:10.1086/302205.

Tables and Figures

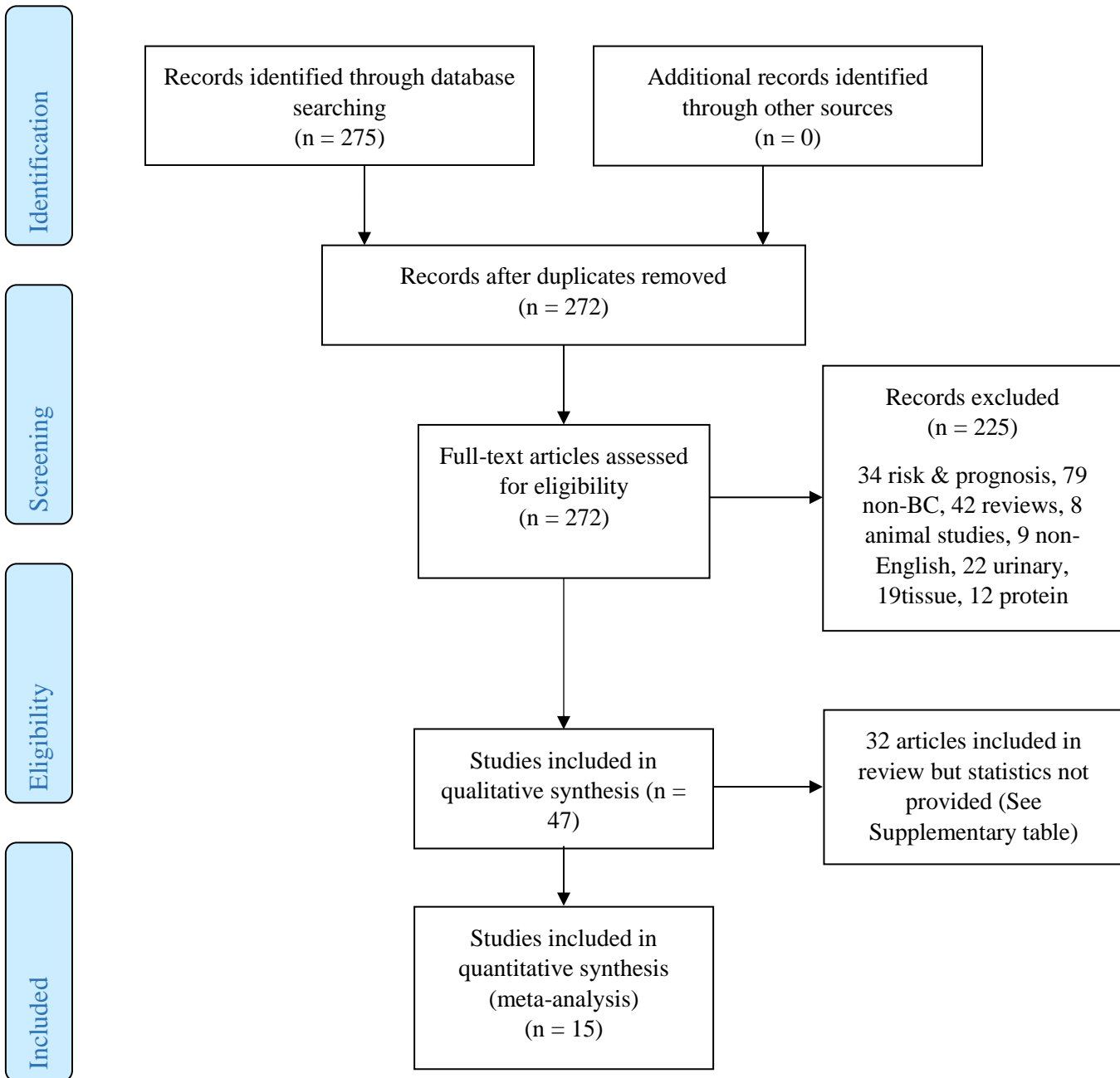


Figure 1: PRISMA Flow diagram summarising the systematic review process

No.	Authors	Cancer type	Patients	Controls	Fluid	Method	Regions of interest	Sensitivity	Specificity	AUC	AUC 95% CI	Validation cohort
miRNA												
1	Yang et al.[32]	all BC	168 BC, paired 40 samples post op, 30 unpaired recurrences	104 normal	serum	qRT-PCR	miR-210	97.6%	69.2%	0.898	0.855–0.931	No
2	Du et al.[64]	all BC (TCC)	42 NMIBC, 14 MIBC	60 normal	plasma	qRT-PCR	miR-497					Yes
							miR-663b					Yes
							miR-497 and miR-663b					Yes
3	Tölle et al.[34]	all BC, stats for >Ta (TCC)	18 Ta, 20 >Ta	20 normal	whole blood	qRT-PCR	miR-26b-5p	65.0%	94.1%	0.824	0.663-0.929	No
							hsa-miR-144 5p	70.0%	82.4%	0.779	0.613-0.899	No
							hsa-miR-374-5p	60.0%	94.0%	0.774	0.606-0.894	No
4	Jiang et al.[35]	all BC	10 NMIBC, 10 MIBC	10 normal	serum	qRT-PCR	panel of 6 miRNAs §	90.0%	90.0%	0.956	0.922–0.978	Yes
5	Scheffer et al.[69]	all BC	11 NMIBC, 11 MIBC	10 benign	serum	qRT-PCR	miR-141			0.714	0.519–0.910	Yes
						qRT-PCR	miR-639			0.752	0.571–0.934	Yes
6	Adam et al.[36]	all BC (TCC)	10 MIBC, 10 NMIBC	18 benign + normal	plasma	qRT-PCR	panel of 40 miRNAs*	90.0%	89.0%	0.91		No
RNA												
7	Osman et al.[39]	All BC (TCC + cc)	18 NMIBC, 26 MIBC + metastatic	29 normal	plasma	qRT-PCR	panel of 7 gene mRNAs∞	83.0%	93.0%	0.901	0.803-0.960	No
8	Ismail et al.[37]	all BC (TCC and SCC)	30 NMIBC, 90 MIBC	30 normal	serum	qRT-PCR	S100A4 gene	90.0%	92.0%	0.978		No
							S100A6 gene	86.7%	84.0%	0.924		No
							S100A7 gene	73.3%	93.3%	0.895		No
							S100A8 gene	85.0%	92.0%	0.935		No
							S100A9 gene	81.7%	92.0%	0.944		No
							S100A11 gene	83.3%	91.0%	0.934		No
cfDNA												
9	Hauser et al.[41]	all BC	75 NMIBC, 20 MIBC	53 normal, 31 benign, 48 resected	serum	qPCR	ACTB-106	91.6%	43.3%	0.686	0.617-0.755	No
10	Ellinger et al.[40]	RC cohort (TCC and SCC)	UCC: 12 NMIBC, 30 MIBC SCC: 3 MIBC	45 BPH	serum	qPCR	PTGS2 gene	95.6%	62.2%	0.836	0.753–0.918	No
11	von Knobloch et al.[68]	all BC (TCC)	37 NMIBC, 21 MIBC	20 normal	serum	PCR based MSA	panel of 17 microsatellites†	80.3%	80.0%			No
12	Hauser et al.[61]	all BC	75 NMIBC, 20 MIBC	45 TURB no BC,	serum	Methylation specific PCR	panel of 9 hypermethylated segments‡	62.1%	88.7%	0.825	0.761-0.890	No
13	Ellinger et al. [62]	RC cohort (TCC and SCC)	UCC: 12 NMIBC, 30 MIBC SCC: 3 MIBC	45 PCa	serum	Methylation specific PCR	APC, GSTP1 or TIG1	80.0%	93.3%	0.867	0.785–0.948	No
							APC	59.5%	100%	0.798	0.698–0.897	No
							DAPK	2.4%	100%	0.3512	0.385–0.638	No
							GSTP1	59.1%	93.2%	3.777	0.676–0.877	No
							PTGS2	24.4%	100%	0.3622	0.506–0.739	No
							TIG1	31.8%	100%	0.659	0.536–0.782	No

							GSTP1 or TIG1	66.7%	93.3%	0.800	0.704–0.895	No
							GSTP1 or APC	75.6%	93.3%	0.844	0.757–0.931	No
							TIG1 or APC	68.9%	100%	0.844	0.757–0.931	No
							TIG1, GSP1, APC or PTGS2	80.0%	93.3%	0.867	0.785–0.948	No
14	Valenzuela et al.[43]	all BC (TCC and SCC)	82 TURBTs, 4 RC	31 normal, 18 benign	serum	methylation specific PCR	p16(INK4a) promoter gene methylation	23.0%	98.0%			No
CTCs												
15	Qi et al.[44]	All BC	20 BC	23 normal	blood	Ligand PCR	Folate receptor alpha	82.1%	61.9	0.819	0.738-0.883	No

Table 1: Summary of included literature with sensitivity, specificity and/or AUC

hsa-miR-92b, hsa-miR-1826, hsa-miR-92b-AS, hsa-miR-33b, hsa-miR-1246, hsa-miR-1290, hsa-miR-1268-AS, hsa-miR-1914, hsa-miR-923-P, hsa-miR-23a, hsa-miR-923, hsa-miR-1469-AS, hsa-miR-184-P, hsa-miR-219-1-3p, gender=male, hsa-miR-25, hsa-miR-935, hsa-miR-23b, hsa-miR-92a, hsa-miR-1228*-AS, hsa-miR-520c-3p-AS, hsa-miR-566-P, hsa-miR-33a-AS, hsa-miR-1254, hsa-miR-1181, hsa-miR-155*MM1T/C, hsa-miR-487a, hsa-miR-1273, hsa-miR-541, hsa-miR-195*, hsa-miR-487b, hsa-miR-148b, hsa-miR-634, hsa-miR-155MM1G/A, hsa-miR-1197, hsa-miR-546h, hsa-miR-32, hsa-miR-720, hsa-miR-202-AS, hsa-miR-937-AS
 ⌘D5S1720, D5S476, D8S261, D8S560, D9S171, D9S925, D9S15, D13S153, D14S750, D14S61, D14S267, D17S799, D17S1306, D20S486, D20S607, D20S481, and D20S480
 §miR-152, miR-148b-3p, miR-3187-3p, miR-15b-5p, miR-27a-3p, miR-30a-5p
 ‡TIMP3, APC, RARB, TIG1, GSTP1, p14, p16, PTGS2 and RASSF1A
 ∞IGFBP7, SNX16, CSPG6, CTSD, CHD2, NELL2, TNFRSF7

Study details			Discovery cohort						Validation cohort					
Literature	Cancer type	Fragments	Patients	Controls	Sensitivity	Specificity	AUC	AUC 95% CI	Patients	Controls	Sensitivity	Specificity	AUC	AUC 95% CI
Du et al.[64]	all BC	miR-497	56	60				not given	109	115			0.694	0.624-0.764
	all BC	miR-663b	56	60				not given	109	115			0.577	0.501-0.653
	all BC	miR-497 and miR-663b	56	60				not given	109	115	69.7%	69.6%	0.711	0.641-0.780
Jiang et al.[35]	all BC	panel of 6 miRNAs §	120	120	90.0%	90.0%	0.956	0.922–0.978	110	110	80.0%	89.1%	0.899	0.851–0.936
Scheffer et al.[69]	all BC	miR-141	22	10			0.714	0.519–0.910	126	105				
	all BC	miR-639	22	10			0.752	0.571–0.934	126	105				

Table 2: Summary of included literature with validation cohorts

§miR-152, miR-148b-3p, miR-3187-3p, miR-15b-5p, miR-27a-3p, miR-30a-5p