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Systematic or Meta-analysis Studies

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

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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.

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 cm^3. 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

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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 subclones 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 Fig. 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 Fig. 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 studied with a median of 56 (IQR 41-90) patients recruited. There was variability in reporting matched controls and validation sets. A summary of findings