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DOI:
10.1016/j.nhtm.2016.12.001

Document Version
Peer reviewed version

Citation for published version (Harvard):
D'Costa, J, Ward, D & Bryan, R 2017, 'Urinary biomarkers for the diagnosis of urothelial bladder cancer', New Horizons in Translational Medicine, vol. 3, no. 5, pp. 221-223. https://doi.org/10.1016/j.nhtm.2016.12.001

NHTM_40

URINARY BIOMARKERS FOR THE DIAGNOSIS OF UROTHELIAL BLADDER CANCER

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Keywords:

Bladder cancer; urinary biomarkers; diagnosis.

ABSTRACT

Urothelial bladder cancer is a common cancer associated with considerable burden for both patients and healthcare providers alike. The majority of patients present with non-muscle-invasive bladder cancer (NMIBC) which, although not immediately life-threatening, requires appropriate initial management and long-term surveillance which is both invasive and costly. Accurate diagnostic urinary biomarkers could be transformational in this setting, yet have proved to be a significant challenge to bladder cancer scientists over the last two decades. Such biomarkers would need to represent a range of tumour grades and stages, encompass inter- and intra-tumour heterogeneity, and compete with the current diagnostic gold standard of cystoscopy with a sensitivity and specificity of 85% and 87%, respectively. For the field to move forward in this current exciting era of high-throughput proteomics and genomics, bladder cancer scientists need to find a consensus on the optimal urinary substrate (DNA, RNA, protein, etc) and deliver robust well-designed studies in the correct populations with appropriate statistical input. Issues relating to tumour heterogeneity and anticipatory diagnosis also require considerable thought. The challenge remains unchanged.

FOCAL POINTS

- Accurate urinary biomarkers for the diagnosis of urothelial bladder cancer could be transformational for patients and healthcare providers alike.
- To date, despite several FDA approvals, no such markers are routinely used in the clinical setting.
- Poor study design and non-representative study populations are major contributory factors and recent systematic reviews have highlighted such weaknesses.
- Low grade and low stage tumours are common, yet the most difficult to diagnose non-invasively; however, they should be incorporated into study populations in proportions representative of the incident and/or recurrent disease patient setting to avoid bias.
- Promising urinary biomarker substrates include proteins and nucleic acids, with inherent strengths and weaknesses.
- Enduring challenges remain inter- and intra-tumour heterogeneity, and anticipatory diagnosis.

COMMENTARY

Urothelial bladder cancer (UBC) is the seventh commonest cancer in Western societies [1], resulting in 69,000 and 180,000 new cases per year in the USA and EU, respectively. The vast majority of new cases are diagnosed following single or repeated episodes of haematuria (blood in the urine) which is investigated by cystoscopy (inserting a “telescope” via the urethra into the bladder) and around 10% of patients investigated for haematuria will be diagnosed with UBC [2]. Following initial treatment by transurethral resection of bladder tumour, 75-85% of these patients will be diagnosed with non-muscle-invasive tumours (NMIBC, stages pTa/pT1/pTis), and the remainder muscle-invasive tumours (MIBC, stages pT2-4) [3]. Thereafter, treatment strategies differ markedly: patients with MIBC are likely to undergo more radical therapy with combinations of chemotherapy and radiotherapy or cystectomy (removal of the bladder) [4], whereas those with NMIBC will be treated with intravesical therapy (therapies delivered into the bladder) followed by cystoscopic surveillance (regular inspection of the bladder) [5]. Schedules of cystoscopic surveillance (and the nature of intravesical therapy) are determined by the risk category of NMIBC (low-, intermediate- or high-risk) [5]. With disease recurrence a lifetime risk across all NMIBC categories (up to 80% [6]), and progression to MIBC an important consideration for high-risk NMIBC patients (up to 45% [6;7]), cystoscopic surveillance represents the mainstay of longer term management for all NMIBC patients. Urine cytology is often used as an adjunct to cystoscopy: the microscopic detection of cancer cells in the urine is a very specific indicator of UBC but has poor sensitivity for low-grade UBC, resulting in low overall sensitivity [8].

Cystoscopy is invasive and burdensome for patients and expensive for healthcare providers [9;10], such that from diagnosis to death on a per patient basis UBC is one of the most expensive malignancies to manage [11]. Therefore, non-invasive or urinary biomarkers for the accurate and reliable detection of urothelial bladder cancer (UBC) have the potential to be transformational for both UBC patients and healthcare providers by reducing reliance on cystoscopy for diagnosis and surveillance. Furthermore, this setting is fertile yet challenging ground for translational medicine.

Since UBCs are in direct contact with urine, urine is considered to be a promising biospecimen for developing non-invasive tests to detect and characterise bladder tumours. However, UBCs are highly heterogeneous with high mutational burden and variable copy number aberrations and gene expression profiles [12;13]; thus, different tumours may release different biomarkers (necessitating multimarker tests), and early-stage and low-grade tumours may only release very small amounts of such markers, potentially impairing test sensitivity [14]. Markers must also be highly tumour-specific so that haematuria itself and other non-malignant conditions do not generate false positives [14;15]. In the search for better urinary biomarkers genomic, proteomic and metabolomic approaches have all yielded promising results [14;16-19]. Despite such work over several decades [20], a 2015 WHO/ICUD consensus stated that [8]:

- *Despite considerable advances in recent years, the authors feel that at this stage the added value of molecular markers for the diagnosis of urothelial tumors has not yet been identified.*
- *Current data suggest that some of these markers may have the potential to play a role in screening and surveillance of bladder cancer.*
- *Well-designed protocols and prospective, controlled trials will be needed to provide the basis to determine whether integration of molecular markers into clinical decision-making will be of value in the future.*

We recently undertook a systematic review of diagnostic and prognostic urinary protein biomarkers and formed similar conclusions [20], principally that:

- *The majority of urine biomarker studies contain bias or are insufficiently reported.*
- *The urinary concentrations of a large number of proteins are increased by the presence of bladder cancer, but most proteins are not increased in all cases and are not specific to bladder cancer.*
- *NMP22, BTA, UBC and Cyfra 21-1 are the only well-validated urinary protein biomarkers and their sensitivities and specificities are well below those of cystoscopy.*

We considered our approach to this systematic review to be stringent yet pragmatic [20], such that it would provide a useful resource for workers in the field. We applied a number of criteria to define whether individual studies provided "equivocal" or "unequivocal data" regarding a particular biomarker(s) [20]. Unequivocal data were generated by studies which comprised of ≥20 cancer patients and ≥20 controls; sensitivity and specificity had to be reported. Importantly, we also required unequivocal studies to comprise ≥25% stage pTa tumours (generally, smaller tumours and more difficult to detect non-invasively, and whose incidence is c.50% [3;21]) and ≥15% grade 1 tumours (the least cellularly and molecularly abnormal tumours [13] so also difficult to detect, and whose incidence is c.25% [21]). These parameters ensured that the selected unequivocal studies had to possess an element of statistical relevance, and also be representative of a normal UBC patient population. Furthermore, if unequivocal data were generated from ≥3 studies, then we considered the biomarker data to be validated.

We also classified the identified proteins as either "possible" or "unlikely" biomarkers dependent upon whether the combined sensitivity and specificity was ≥80% or <80%, respectively. White light cystoscopy is currently the gold standard detection method for UBC, the reported sensitivity and specificity of which vary greatly but a 2012 meta-analysis arrived at values of 85 and 87%, respectively [22]; any urinary biomarker would need to match or improve upon cystoscopy to be acceptable to patients and urologists. Hence, we were permissive in our definition of a possible biomarker. Yet, as described, very few studies could be considered as unequivocal, although these studies did report several possible biomarkers: fibronectin, clusterin, CEACAM1, apolipoprotein A4, calprotectin, CD147, coronin-1A, DJ-1, reg-1, stathmin-1, and γ-synuclein [20].

We specifically limited our review to soluble urinary proteins as historically this has been the main focus of UBC urinary biomarker research. Additionally, with the technology currently available, they are the easiest class of biomolecule to use for point-of-care testing or to combine in an economical single multiplex assay for the detection of UBC (should a suitable biomarker panel be determined).

We also envisage that measuring volatile metabolites [23], or advances in DNA sequencing may allow point-of-care testing in the not too distant future. In fact, recent publications make a strong case for DNA-based biomarkers being the frontrunners in the race to reduce reliance on cystoscopy [24-28]. Although the amount of DNA that can be extracted from urine is low and variable, PCR and advanced analysis techniques such as next generation sequencing allow identification of tiny amounts of tumour DNA in the majority of urine samples, even in the presence of an excess of non-tumour DNA [27]. Genome wide copy number changes in urinary DNA, microsatellite analysis, methylation and mutations have all been used for the purpose [24-28]. Studies of urinary DNA have focussed almost exclusively on DNA extracted from the urinary cell-pellets obtained by centrifuging urine; however, we and others have found that cell-free DNA (cfDNA) in the urine supernatant contains a higher fraction of tumour DNA than cell pellet DNA, and we are optimistic that urinary cfDNA could underlie a clinically useful test for UBC detection [24;29]. As with protein biomarkers, the performance of DNA biomarkers requires thorough evaluation prior to clinical uptake, particularly in the disease surveillance setting.

Whatever the biomarker substrate (proteins, nucleic acids, etc) or source (urine supernatant, cell pellet, etc), the field now needs to concentrate on designing and delivering the right studies in the right patient populations and with due statistical consideration so that evidence synthesis is robust, results are reproducible, and product marketing is not premature. Issues such as inter- and intra-tumour heterogeneity should also be addressed, which may require the utilisation of biomarker panels comprised of 10s or100s of individual markers [19;24]. And the conundrum of “anticipatory” or “pre-emptive” diagnosis requires clarification - the scenario whereby a patient is urinary biomarker positive and cystoscopy negative, yet who develops recurrence within the following 12-24 months. Should such patients be treated as false positive, be placed under closer surveillance, be the subject of personalised biomarkers based upon the tumour’s biomarker expression, or even be treated pre-emptively with intravesical therapies? If the biomarker is highly specific, then the latter three options could all be appropriate. The future is exciting and challenging.

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Figure 1: A suggested urothelial cancer biomarker research pipeline