Optimising the investigation of haematuria and bladder cancer

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DECLARATION

“I, Wei Shen Tan, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. Where assistance and materials have been obtained, I have acknowledged as appropriate”

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THESIS ABSTRACT

There remains a lack of consensus between guidelines on which haematuria patients should be investigated. In this doctoral thesis, I report the contemporary incidence of urinary tract cancer in haematuria patients recruited to a multi-centre study. I showed that the current National Institute for Health and Care Excellence (NICE) guidelines would miss 3.7% of cancers and that NVH warrants investigation due to the risk of high risk bladder cancer.

I subsequently reported that ultrasound can safely replace CT urogram for the imaging of the upper urinary tracts in NVH patients despite a low sensitivity of upper tract urothelial cancer (UTUC) because of a very low (0%) incidence of UTUC. Analysis of urine cytology suggests that it has a poor sensitivity for the detection of bladder cancer and UTUC and a high risk of false positive result.

My systematic review of urine-based biomarkers for the detection of bladder cancer indicates that multi-target panels have a better diagnostic performance although no biomarker has been prospectively validated in a clinical trial. An interim analysis of the diagnostic performance of the UroMark assay in a prospective study report a sensitivity of 87.5% with a negative predictive value of 92.9%.

I subsequently developed and validated a nomogram to guide patient selection for haematuria investigation. The haematuria cancer risk score (HCRS) approach identified more urinary tract cancers compared to the current NICE guidance.

Finally, I utilised a mixed method approach and reported that >75% of patients would accept a urine-based biomarker with a minimum sensitivity of 90% in the

non-muscle invasive bladder cancer surveillance setting. Direct visualisation of bladder cancer is a key feature of cystoscopy which patients hold in high regard.

In conclusion, these findings are important and will assist the development of future haematuria guidelines both in terms of patient selection and choice of diagnostic tests. It also offers guidance to other research groups in biomarker discovery who are planning future biomarker validation studies.

IMPACT STATEMENT

Haematuria is a common symptom observed by both primary care physicians and urologists. However, there remains a lack of consensus between guidelines on which patients with haematuria should be investigated.

In this doctoral thesis, I reported the contemporary incidence of urinary tract cancers in patients with haematuria in the UK. This represents the first multi-centre study which accurately captures the incidence of urinary tract cancers in patients with haematuria who were referred to secondary care in the UK.

I subsequently reported that it is safe to substitute CT urogram with renal, bladder ultrasound (RBUS) when imaging the upper urinary tracts of patients with non-visible haematuria (NVH). This would reduce patient exposure to ionising radiation and minimise the risk of contrast allergic reaction without compromising on risk of missing upper tract cancers. While this is already practiced in some centres in the UK, my results for the first time provides evidence to inform the use of RBUS in the NVH setting. I also report that urine cytology should not be routinely performed as part of haematuria investigations due to the risk of false positive results which will subject patients to further unnecessary invasive procedures. The current NICE guidelines do not comment on the type of upper tract imaging recommended and the role of urine cytology. I anticipate my results will help inform recommendations in the expected 2019 NICE guidance.

I subsequently performed an interim analysis of a validation study to determine the utility of a novel urine-based biomarker to identify bladder cancer, the UroMark. Once the validation study is complete, the UroMark may promote early evaluation of haematuria, potentially reducing the delay in cancer diagnosis and offering a non-invasive approach for the evaluation of haematuria.

This was followed by the development of a nomogram, which was externally validated to optimise the selection of patients who will benefit from investigations following a presentation of haematuria. The nomogram which utilised patient age, type of haematuria, smoking history and gender performed better than current haematuria guidelines which utilised only age and type of haematuria. This represents the first externally validated nomogram developed to guide which patients with visible or non-visible haematuria should be investigated. Adoption of this nomogram based approach would reduce the number of patients subjected to investigations while optimising the detection rate of patients at risk of urinary tract cancers. Future work would involve validation in the primary care setting before adoption by general practitioners.

Finally, I assessed patients' view by postal questionnaire survey and semi-structured telephone interviews on the use of a urinary biomarker and cystoscopy, along with their experience of being diagnosed with bladder cancer. This study represents the first study to qualitatively assess reasons for patient decision making relating to a non-invasive diagnostic test. This will be useful as a benchmark for researchers to determine patient requirement for such a test before acceptance.

Chapters of this thesis comprise of 9 published manuscripts. The publications have been particularly well received with three publications within the top 5% of research tracked by Altmetric and one within the top 10%. One of my presentations was selected as best poster in the European Association of Urology 2019 meeting. My results provide both clinicians and patients information which will be useful for counselling and may guide their decision making on the requirement for haematuria investigations. It provides guidance on patient

selection and combination of diagnostic tests which should be used for the investigation of haematuria. It will no doubt be relevant in the development of future international haematuria guidelines.

ACKNOWLEDGMENTS

I would like to express my gratitude and thanks to my supervisors, Prof John Kelly and Dr Andrew Feber, for the guidance and mentorship over the last 8 years. I treasure the opportunity to work on the DETECT studies as well as other projects which I have embarked on over the years. I am grateful for their insightful discussions, ideas and advice which has helped me to develop a critical academic mindset which will be my foundation for a future academic urology career.

I would like to thank the other members of the Kelly-Feber research team. I am grateful to Dr Liqin Dong for running the UroMark assay using extracted urinary DNA and Miss Sheida Rezaee for extracting most the DNA from urine samples. Other members of the team include Dr Pramit Khetrapal, Dr Patricia deWinter, and Dr Ron Finn who assisted in a magnitude of ways, some of which includes preparation of urine tubes, processing of urine and other administrative tasks.

I would also like to express my thanks to the Surgical and Interventional Trials Unit (SITU), UCL, particularly to Miss Rachael Sarpong, Ms Chris Brew-Graves and Mr Norman Williams who have been instrumental in the development of the study, guiding me through the IRAS paperwork and subsequently HTA approval as well as other administrative tasks.

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ACHIEVEMENTS DURING PHD

List of awards during PhD

2019 European Association of Urology- **Best poster (Urine, serum and tissue diagnostic innovations in urothelial cancer session)**

2019 European Association of Urology- **Best poster (Outcomes of partial nephrectomy: Kidney function and beyond)**

2018 Royal Society of Medicine Urology Section & BAUS Section of Academic Urology- **BAUS Academic Research Prize Nominee**

2018 Royal Society of Medicine Urology Section & BAUS Section of Academic Urology- **Malcolm Coptcoat Prize Nominee**

2017 American Urology Association- **Best Abstract (Bladder Cancer: Basic Research & Pathophysiology I)**

2016 Enhanced Recovery after Surgery Society (UK)-**2nd Prize for Best Paper**

2016 Asia Congress of Urology- **Best Abstract (Oncology)**

2016 17th Annual Network for the Advancement of Patient Blood Management, Haemostasis and Thrombosis (NATA) Symposium- **Best Academic Paper Nominee**

List of funding and grants awarded during PhD

2018 **Robert Brown Travel Award**- £300 (Sole applicant)

2017 **Mason Medical Research Fellowship**- £44,563 (Sole applicant)

2016 **The Urology Foundation Research Scholarship**- £81,839 (Sole applicant)

2016-2018 **UCL Division of Surgery & Interventional Sciences** Studentship

List of national/ international presentations relating to this PhD

I gave 29 presentations during my PhD including 6 invited talks, three of which were plenary presentations. The presentations below represent presentations relating to this PhD.

March 2019	**Mix methods approach to explore patients' perspectives on the acceptability of a urinary biomarker test in replacement of cystoscopy in bladder cancer surveillance.** EAU 2109, Barcelona- Moderated poster
March 2019	**Development and validation of a haematuria cancer risk score to identify patients at risk of harbouring cancer.** EAU 2109, Barcelona- Moderated poster
June 2018	**Haematuria: Controversies in Everyday Practice- Can CT intravenous urogram be replaced with renal tract ultrasound for non-visible haematuria?** BAUS 2018- Invited plenary lecture
June 2018	**Who should be investigated for haematuria? A prospective observational study of 3556 patients**. BAUS 2018, Liverpool- Moderated poster
June 2018	**Can CT intravenous urogram be replaced with renal tract ultrasound for non-visible haematuria?** BAUS 2018, Liverpool- Moderated poster
May 2018	**Can renal tract ultrasound replace CT urography for the evaluation of microscopic haematuria? Results of a prospective observational study.** AUA 2018, San Francisco- Moderated poster
May 2018	**Who should be evaluated for haematuria? A comparison of international guidelines.** AUA 2018, San Francisco- Moderated poster
May 2018	**Does urinary cytology have a role in haematuria investigations? Results of a prospective observational study (DETECT I).** AUA 2018, San Francisco- Moderated poster
April 2018	**Who should be investigated for haematuria? Results of a contemporary prospective observational study of 3556 patients.** RSM Urology Section & BAUS Section of Academic Urology- Oral presentation

April 2018 **Can renal tract ultrasound replace CT urogram in patients investigated for non-visible haematuria?** RSM Urology Section & BAUS Section of Academic Urology- Oral presentation

April 2018 **Does urinary cytology have a role in haematuria investigations? Results of a prospective observational study.** RSM Urology Section & BAUS Section of Academic Urology- Poster presentation

June 2016 **A New Urine Test for the Haematuria Clinic.** BAUS Oncology 2016, Cardiff, UK- Invited plenary presentation.

June 2016 **Low INPP4B expression predicts poor prognosis in locally advanced and metastatic bladder cancer**. BAUS 2016, Liverpool, UK- Moderated poster.

List of publications relating to this PhD

I have co-authored a total of 39 manuscripts and a book chapter during my PhD, of which, I was first author for 24 manuscripts. The publications below represent publications relating to this PhD.

1. Tan WP, Kelly JD, **Tan WS**. **Bladder cancer and haematuria.** *Trends Urol Men Health*. 2018 (In press).

2. **Tan WS**, Teo CH, Chan D, Heinrich M, Feber A, Sarpong R, Allan J, Williams N, Brew-Graves C, Ng CJ, Kelly JD; DETECT II trial collaborators. **Mix methods approach to explore patients' perspectives on the acceptability of a urinary biomarker test in replacement of cystoscopy in bladder cancer surveillance**. *BJU Int.* 2019 Jan 29. doi: 10.1111/bju.14690. PMID: 30694612

3. **Tan WS**, Ahmad A, Feber A, Mostafid H, Cresswell J, Fankhauser CD, Waisbrod S, Hermanns T, Sasieni P, Kelly JD, DETECT II trial collaborators. **Development and validation of a haematuria cancer risk score to identify patients at risk of harbouring cancer.** *J Intern Med.* 2018 Dec 6. doi: 10.1111/joim.12868. PMID: 30521125.

4. **Tan WS**, Sarpong R, Khetrapal P, Rodney S, Mostafid H, Cresswell J, Watson D, Rane A, Hicks J, Hellawell G, Davies M, Srirangam SJ Dawson L, Payne D, Williams N, Brew-Graves C, Feber A, Kelly JD, on behalf of DETECT I trial collaborators. **Does urinary cytology have a role in haematuria investigations?** *BJUI Int* 2018 Jul 12. doi: 10.1111/bju.14459. PMID: 30003675.

5. **Tan WS**, Tan WP, Tan MY, Khetrapal P, Dong Liqin, deWinter P, Feber A, Kelly JD. **Novel urinary biomarker for the detection of bladder cancer: A systematic review**. *Cancer Treat Rev.* 2018 May 29;69:39-52. PMID: 29902678.

6. **Tan WS**, Sarpong R, Khetrapal P, Rodney S, Jalil R, Mostafid H, Cresswell J, Hicks J, Rane A, Henderson A, Watson D, Cherian J, Williams N, Brew-Graves C, Feber A, Kelly JD, on behalf of DETECT I trial collaborators. **Can renal and bladder ultrasound replace CT urogram in patients investigated for microscopic haematuria**. *J Urol.* 2018 Apr 24. pii: S0022-5347(18)43045-5. PMID: 29702097.

7. **Tan WS**, Feber A, Sarpong R, Khetrapal P, Rodney S, Jalil R, Mostafid H, Cresswell J, Hicks J, Rane A, Henderson A, Watson D, Cherian J, Williams N, Brew-Graves C, Kelly JD, on behalf of DETECT I trial collaborators. **Who should be investigated for haematuria? Results of a contemporary prospective observational study of 3556 patients**. *Eur Urol*. 2018 Apr 10. pii: S0302-2838(18)30184-2. PMID: 29653885.

8. **Tan WS**, Tan WP. **Urinary biomarker for the detection of recurrence following non-muscle invasive bladder cancer: Are we there yet?** *Transl Androl Urol*. 2017 doi: 10.21037/tau.2017.12.18. PMID: 29644175.

9. **Tan WS**, Feber A, Dong L, Sarpong R, Razaee S, Rodney S, Khetrapal P, de Winter P, Ocampo F, Jalil R, Williams N, Brew-Graves C, Kelly JD. **DETECT I & DETECT II: A study protocol for a prospective multicentre observational study to validate the UroMark assay for the detection of bladder cancer from urinary cells**. *BMC Cancer*. 2017 Nov 15;17(1):767. PMID: 29141603.

10. Feber A, Dhami P, Dong L, de Winter P, **Tan WS**, Martinez-Fernande M, Paul D, 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, Kelly JD. **UroMark - A urinary biomarker assay for the detection of bladder cancer**. *Clin Epig*. 2017 Jan 31;9:8. PMID: 28163793

11. **Tan WS**, Rodney S, Lamb BW, Feneley M, Kelly JD. **Management of non-muscle invasive bladder cancer: A comprehensive analysis of guidelines from the United States, Europe and Asia**. *Cancer Treat Rev*. 2016 May 10;47:22-31. PMID: 27231966.

CONTENTS

Abbreviations

AUA	American Urology Association
AUC	Area under the curve
BAUS	British Association of Urological Surgeons
BCG	Bacillus Calmette-Guerin
BIPQ	Brief Illness Perception Questionnaire
CI	Confidence interval
CIS	Carcinoma in situ
CT	Computed tomography
CT KUB	CT kidney, ureters, bladder
CTU	CT urogram
CUETO	Club Urológico Español de Tratamiento Oncológico
DNA	Deoxyribonucleic acid
EAU	European Association of Urology
eCRF	Electronic case record form
ELISA	Enzyme-linked immunosorbent assay
EMR	Electronic medical records
EORTC	European Organization for Research and Treatment of Cancer
FFPE	Formalin-Fixed Paraffin-Embedded
FISH	Fluorescence in-situ hybridization
GSTM1	Glutathione S-transferase M1
HR	Hazard ratio
HTA	Health technology assessment
ICGC	International Cancer Genome Consortium
ICUD	International Consultation on Bladder Cancer
JUA	Japan Urology Association
LUTS	Lower urinary tract symptoms
MAS	Minimal acceptable sensitivity
MDT	Multidisciplinary team
MIBC	Muscle invasive bladder cancer
MMC	Mitomycin C
NAT2	N-acetyltransferases 2
NBI	Narrow band imaging

NCCN	National Comprehensive Cancer Network
NGS	Next generation sequencing
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NMIBC	Non-muscle invasive bladder cancer
NPV	Negative predictive value
NVH	Non-visible haematuria
OS	Overall survival
PBS	Phosphate buffered saline
PCR	polymerase chain reaction
PDD	Photodynamic diagnostic
PPV	Positive predictive value
PUNLMP	Papillary urothelial proliferation of low malignant potential
RBC	Red blood cell
RBUS	Renal, bladder ultrasound
RFS	Recurrence free survival
ROC	Receiver operating characteristics
RT-PCR	Reverse transcription polymerase chain reaction
SCC	Squamous cell carcinoma
SWOG	Southwest Oncology Group
TCGA	The Cancer Genome Atlas
TURBT	Transurethral resection of bladder tumour
UCC	Urothelial cell carcinoma
UCL	University College London
UK	United Kingdom
US	United States
UTI	Urinary tract infection
UTUC	Upper tract urothelial carcinoma
VH	Visible haematuria
WHO	World Health Organisation

Tables

Figures

CHAPTER 1 : INTRODUCTION

1.1 Haematuria

Haematuria represents a common urinary symptom in primary care. It is classified as visible haematuria (VH) and non-visible haematuria (NVH). VH is a clinical symptom which is widely regarded as a red flag and fulfils the National Health Service (NHS) 2 week wait suspected cancer referral guidelines for investigations due to the risk of urinary tract cancer (2). VH is often alarming and of great concern to most patients particularly if symptoms persist. A diagnosis of malignancy should be considered until proven otherwise in patients presenting with VH, although it is acknowledged that the majority of these patients will not have cancer (3). Previous reports suggest that up to 20.9% of patients referred for investigation of VH will harbour urinary tract cancer supporting the rational for prompt investigations (3). The significance of NVH is less clear. It is estimated that 2.5% of patients in the community have NVH (4). Data from secondary care suggests that 5% of patients with NVH will harbour urinary tract cancer. This increases to 18% in male patients age ≥70 years (5, 6).

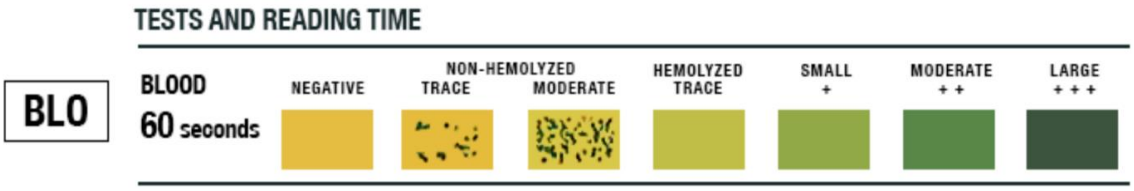
Traditionally, NVH was diagnosed by inverted phase microscopy with positivity defined as ≥3 red blood cells (RBC) per high-power field (7). This is based on historical reports that urine dipstick has a specificity of between 56.9-99.1% for the detection of 2-5 RBC per high-power field despite a sensitivity approaching 100% for the detection of RBC (8, 9). The reported false positives on urine dipstick are attributed to myoglobinuria as well as other oxidising contaminants which can be excluded by urine microscopy.

However, it is worth acknowledging that, urine microscopy may result in a higher than acceptable false negative rate especially in the primary care setting. Delays in processing urine for microscopy results in RBC lysis. It is estimated that the

RBC count falls by up to 9% by five hours and 35% by 72 hours (10). A subsequent systematic review reported that urine dipstick had a positive likelihood ratio of 5.99 (95% confidence interval (CI): 4.04-8.89) and a negative likelihood ratio of 0.21 (95% CI: 0.17-0.26) suggesting it is a good test for NVH in the clinical setting (8).

In the UK, urine dipstick testing, which provides an instant readout for the presence of RBC in urine, is the recommended test for the diagnosis of NVH. Urine microscopy is no longer required to validate results of urine dipstick (11). The use of urinary dipstick analysis is supported by recommendations from a joint working party comprising of the British Association of Urological Surgeons (BAUS) and the Renal Association which led to the introduction of the term NVH (12). In the UK, in the absence of urinary tract infection (UTI), significant asymptomatic NVH is defined as a urine dipstick score of ≥1+ on two or more occasions (Figure 1.1) while symptomatic NVH is defined as urine dipstick score of ≥1 plus lower urinary tract symptoms (hesitancy, frequency, urgency, dysuria) on one occasion (13). Repeating urine dipstick with NVH at least once will reduce the risk of false positive. Trace haematuria on urine dipstick should be considered negative as this likely corresponds to ≤4 RBC per microscopy field (14). However, false positive can occur following ejaculation, dehydration, exercise, menstrual blood and presence of myoglobinuria while high urinary Vitamin C, elevated specific gravity pH <5.1 and proteinuria may result in a false negative test (15-17). In the United States, the term microscopic haematuria is used rather than NVH and diagnosis is by microscopy despite the short comings of microscopy as described above (18).

Figure 1.1: Defining non-visible haematuria using urine dipstick. Adapted from Siemens Healthcare (19).



Haematuria can present in association with lower urinary tract symptoms (LUTS) such as dysuria, frequency or urgency. It is generally recommended that UTI is excluded as a cause for haematuria as it is common in the presence of infection. It is important to understand that significant underlying pathology such as genitourinary malignancy can masquerade as urinary tract infection (UTI) and testing for absence of VH or NVH after treatment is important. In addition, VH and NVH can be associated with pathologies including renal calculi, structural abnormities, haematological, nephrological, iatrogenic, trauma and idiopathic causes.

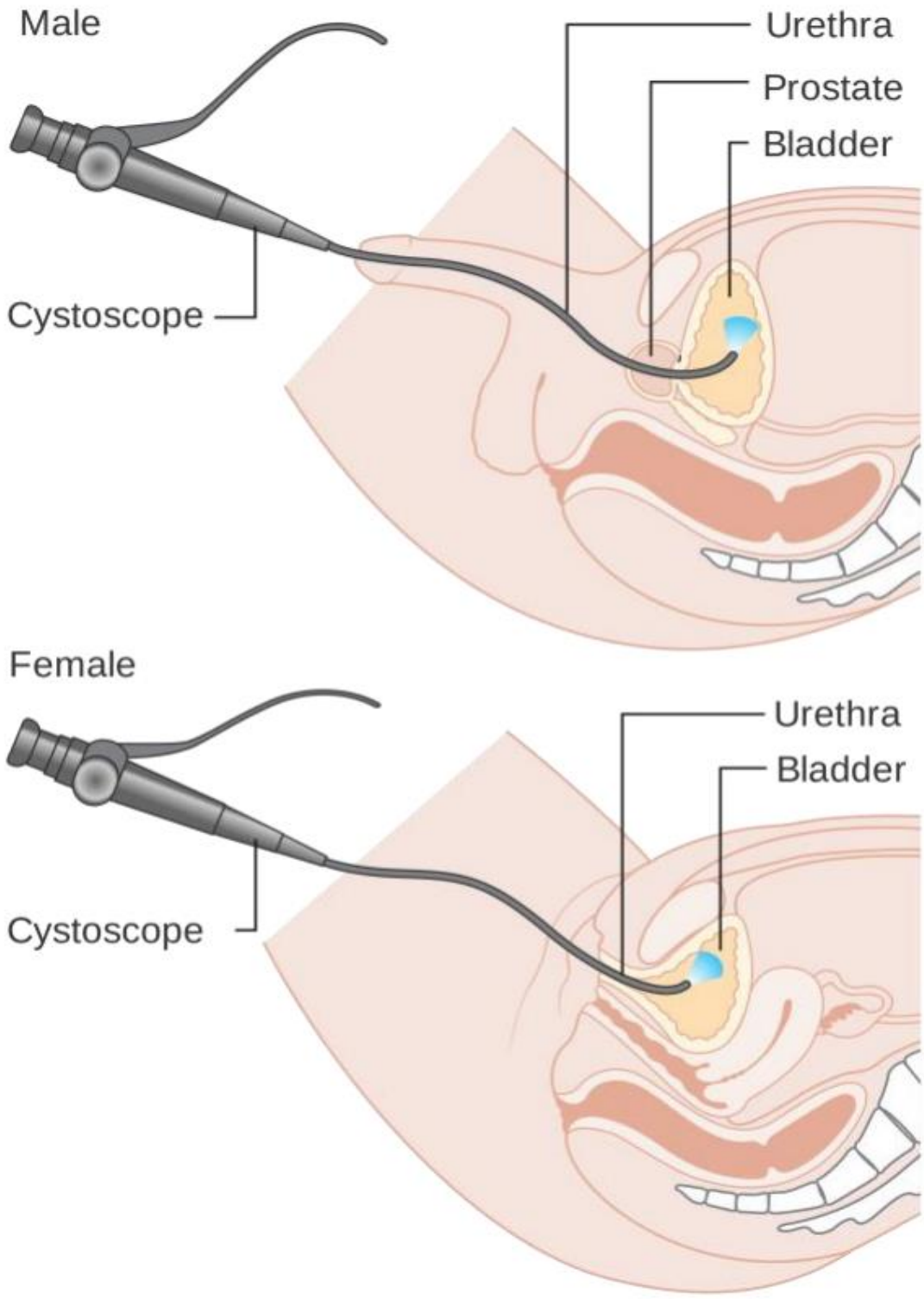
1.1.1 Investigations following a presentation of haematuria

The investigation of haematuria comprises of cystoscopy and upper tract imaging. Flexible cystoscopy enables visualisation of the urethra and bladder using an 18 Fr fibre optic scope and is typically performed following instillation of local anaesthetic infused lubricating jelly into the urethra (Figure 1.2). Cystoscopy is invasive, requires a hospital visit and has up to a 5% risk of a UTI (20). It is estimated that 100,000 cystoscopies are carried out in the UK each year as part of haematuria investigations assuming a 10% incidence of bladder cancer and

based on the fact that approximately 10,000 new bladder cancer cases were diagnosed annually (21).

Imaging of the upper tract is mandatory due to the risk of upper tract cancer which can present with haematuria in the absence of intravesical pathology. Upper tract imaging can be by renal, bladder ultrasound (RBUS), Computed tomography (CT) of kidneys, ureters and bladder (CT KUB) or a CT urogram (CTU). In a series of 1,903 patients evaluated for haematuria, the incidence of upper tract tumours was 0.73% with 28.5% presenting with NVH. RBUS detected 57% (8/14) of upper tract urothelial carcinoma (UTUC) and has a limited role in detecting non-obstructive ureteric tumours (3). Of the six UTUC not detected with renal tract ultrasound, one patient had hydronephrosis which would normally trigger cross sectional imaging. The remaining five patients had a normal finding. Hence, the risk of missing an UTUC by using RBUS instead of CTU is 0.075% (3).

Figure 1.2: Schematic diagram of flexible cystoscopy. Reproduced from BAUS flexible cystoscopy patient information sheet (22).



Based on the poor diagnostic accuracy of renal tract ultrasound in identifying upper tract tumours particularly UTUC, the American Urology Association (AUA) recommends CTU as the upper tract imaging modality of choice in patients with either VH or NVH in the absence of contraindications (23). CTU has a negative predictive value (NPV) of 96% and a positive predictive value (PPV) of 76% (24). The European Association of Urology (EAU) supports the use of RBUS during the initial work-up in haematuria patients but recommends that CTU should be performed following a diagnosis of bladder cancer (25). The National Institute for Health and Care Excellence (NICE) does not specify a recommended form of upper tract imaging (2).

1.1.2 Haematuria guidelines

There remains a lack of consensus among national guideline bodies relating to who should be investigated following a presentation of haematuria in primary care (26). The variation between guidelines is attributed to limited level one or high-quality evidence and such, recommendations are based on data extracted from observational studies. Limitations should be acknowledged when interpreting studies conducted both, in the primary and secondary care setting which are used to formulate guidelines.

Retrospective studies using primary care medical records suggest that only 1.6% of patients aged ≥60 years with NVH had a diagnosis of bladder cancer which is significantly lower than the 9.4% of patients reported in secondary care (3, 27). However, a major bias which is a limiting factor in primary care reports is the inability to differentiate between haematuria attributed to UTI and haematuria

established after UTI was excluded. The former is more common and would not normally trigger haematuria investigation. Secondary care prospective studies are relevant but will invariably have inherent case selection bias (3, 28).

Accepting the limitations set out above, an ideal diagnostic protocol should maximise cancer detection using investigations with a high negative predictive value. However, due to the relatively low incidence of urinary tract cancer (approximately 12.7% based on secondary care data), the negative predictive value of any protocol will be high and can be misleading in the context of optimal tests. In this context, a high test sensitivity is important and interpretation of the negative predictive value and sensitivity is essential. Missing cancers will result in a delay in diagnosis and treatment leading to patient anxiety, a higher risk of disease progression and can have an impact on patient wellbeing reflected as a reduced quality of life (29).

In establishing guidelines, National policy should define what risk threshold should trigger investigation to balance the risk of over investigation, which will subject patients to unnecessary invasive diagnostic tests, resulting in higher healthcare related expenditure with the aim to detect all cancers. Therefore, a risk adapted approach is important to formulate recommendations whereby, all significant cancers and the majority of all cancers are detected in the population most likely to harbour disease. At the same time, the number of investigations should be minimised for patients at low risk of urinary tract cancers to reduce unnecessary testing. Currently, the criteria incorporated into guidelines to determine the need for further investigation for patients presenting with haematuria comprise of age and type of haematuria (VH vs NVH) with some specifying the requirement of LUTS and exclusion of UTI. NICE recommends that

a symptom with an associated risk of harbouring cancer at of at least 3% merits referral whereas the AUA seeks to define the thresholds to detect 99% of all cancers (2, 7).

The variation in haematuria guidelines is highlighted in Table 1.1. NICE guidance recommends that patients ≥45 years with unexplained VH without UTI or persistent VH after successful UTI treatment, as well as patients ≥60 years with unexplained NVH and either dysuria or a raised white cell count on blood test should be referred for a 2-week suspected cancer pathway (2). Non-urgent referral should be considered for patients ≥60 years with recurrent or persistent unexplained UTI. The previous BAUS/ Renal Association haematuria guidelines recommend investigating patients with VH of all ages and patients with asymptomatic NVH aged ≥40 years (12). The AUA recommend that asymptomatic NVH defined as ≥3 RBC per high powered field in voided urine warrant investigation in all patients ≥35 years (23). In contrast, the National Board of Health and Welfare of Sweden has abandoned haematuria investigations for NVH patients but recommends investigating patients presenting with VH (30).

Table 1.1: Differences in haematuria guidelines

Guidelines	Definition of NVH	Time to investigation	Recommendation patient cohort to investigate		Imaging	Requirement for cytology	Follow up
			Visible haematuria	Non-visible haematuria			
American Urology Association, 2016 (23)	≥3 RBC per HPF on microscopy	Not specified	All patients regardless of age	All patients with asymptomatic microscopic haematuria ≥35 yrs. In patients <35 years, cystoscopy can be performed at the discretion of the physician.	CTU for both VH & NVH	Not recommended for asymptomatic NVH. May be used in persistent NVH following a negative work up or in those with carcinoma in situ risk factors (irritative voiding, current/ past tobacco use, chemical exposure) No comment for VH	Annual urinalysis for at least 2 years
National Institute for Health and Care Excellence, 2015 (2)	Not specified	2 weeks/ non- urgent	≥45 years with unexplained VH without UTI	2-week wait referral if: 1) ≥45 years with unexplained NVH without UTI 2) ≥60 years with unexplained NVH with either dysuria OR raised WCC on blood test Non-urgent referral in ≥60 years with recurrent or persistent UTI	Not specified	Not specified Cytology/ urinary biomarker or photodynamic diagnosis/ narrow band imaging at TURBT for patients with suspected bladder cancer	Not specified
British Association of Urological Surgeons, 2008 (12)	≥1+ urine dipstick on ≥2 of 3 samples	Not specified	All patients regardless of age	≥40 years with asymptomatic NVH	Not specified	Not recommended	Monitor for LUTS, VH, proteinuria, declining eGFR, hypertension

Japan Urology Association, 2013 (31)	≥5 RBC per HPF on microscopy	Not specified	≥25 years	≥40 years or ≥1 risk factor*: cystoscopy + upper tract imaging for Other patients: non-invasive screening ɬ	RBUS. CTU following bladder cancer diagnosis	Recommended for VH with cystoscopy NVH patients without risk factors can have cytology/ ultrasound as an alternative to cystoscopy	Retest if VH, LUTS or persistent haematuria. Annual Urinalysis
International Consultation on Urological Diseases, 2011 (32)	Not specified	Not specified	All patients	All patients. Cystoscopy or urinary cytology in patients without risk factors	Not specified	Option instead of cystoscopy in patients without risk factors	Not specified
National Board of Health and Welfare of Sweden 2013 (30)	Not specified	Not specified	All patients	Not recommended	Not recommended in NVH	Not recommended in NVH. No comment for VH.	Not recommended in NVH

*smokers, those exposed to chemical substances, individuals with a prior history of urological diseases, urgency, patients with a history of urinary tract infection, individuals with frequent usage of NSAIDs (especially phenacetin), pelvic organ radiation recipients, and those with a prior history of cyclophosphamide usage

ɬ ultrasound and/ or urinary cytology

Abbreviations: CTU: CT urogram, eGFR: estimated glomerular filtration rate, HPF: high powered field, LUTS: lower urinary tract symptoms, NVH: non-visible haematuria, RBC: red blood cells, RBUS: renal bladder ultrasound, TURBT: transurethral resection of bladder tumour, UTI: urinary tract infection, VH: visible haematuria

1.2 Bladder Cancer

1.2.1 Epidemiology

Bladder cancer is the 8th most common cancer with 429,000 new cases per year diagnosed worldwide (33). In the UK, a total of 10,171 new bladder cancer cases were diagnosed in 2015 which equates to 15.6 per 100,000 persons (21). Globally, Europe has the highest bladder cancer incidence of 17.7 per 100,000 persons, particularly in Southern Europe (21.8 per 100,000 persons) and Western Europe (19.7 per 100,000 persons) (34). Other geographical areas with a high incidence of bladder cancer include North America (19.5 per 100,000 persons) and North Africa (15.1 per 100,000 persons) (34). While urothelial cell carcinoma is the predominant type of bladder cancer, squamous cell carcinoma (SSC) was historically the predominant bladder cancer type in Egypt due to *Schistosoma haematobium* infection. However, public health interventions have led to a decline in such infections and urothelial cell carcinoma (UCC) has largely replaced SCC as the predominant bladder cancer type (35).

Risk factors for bladder cancer can be classified into patient and environmental exposure factors. Patient risk factors include increasing age, sex and genetic alterations. The peak incidence of bladder cancer is between 60- 70 years and it is rare in patients <40 years. The incidence of bladder cancer in males is three-fold higher than in females, which is partially attributed to higher tobacco use in males (36). While genetics play a role in bladder cancer, population studies from national cancer databases suggest that the risk of hereditary factors leading to the development of bladder cancer are low (37). However, it has been postulated that genetic factors may modulate the risk of bladder cancer development following exposure to particular carcinogens. The absence of glutathione S-

transferase M1 (GSTM1) expression, slow acetylation of N-acetyltransferases 2 (NAT2) and short telomeres have been shown to associated with increased risk of bladder cancers in smokers (38-40).

Environmental exposure has been shown to play a significant role in the development of bladder cancer. Cigarette smoking represents the leading cause of bladder cancer. In a large cohort study of 467,528 participants, current smokers (HR 4.06; 95% CI 3.66-4.50) and former smokers (HR 2.22; 95% CI 2.03-2.44) have a higher risk of developing bladder cancer compared to non-smokers (41). Occupational risk factors such as workers exposed to aromatic amines (tobacco, dye, rubber workers, hair dressers, printers, leather workers) and polycyclic aromatic hydrocarbons (waiters, cooks, aluminium workers, seamen, chimney sweeps, petroleum workers) have an increased risk of developing bladder cancer (42). It is estimated that 42% of bladder cancer cases can be prevented based on minimising environmental exposure (21). In fact, the decline in smoking and reduction in occupational risk factors in the UK has resulted in a 39% fall in bladder cancer incidence compared to early 1990s (43).

Other risk factors for bladder cancer include history of previous treatment with pelvic radiotherapy which increases the risk of developing secondary bladder cancer (HR 1.67; 95% CI 1.55- 1.80) (44). The use of drugs such as cyclophosphamide (45) and possibly pioglitazone (46) as well as chronic irritation of the urothelium due to long term catheter use, bladder stones or *Schistosoma haematobium* infection (47).

1.2.2 Classification of bladder cancer

1.2.2.1 Grading of bladder cancer

UCC was previously graded 1-3 according to the 1973 WHO classification (48) which has been superseded by the newer WHO and International Society of Urological Pathology histology classification (49) set out in Table 1.2.

Table 1.2: 1973 and 2004/2006 WHO grading classification

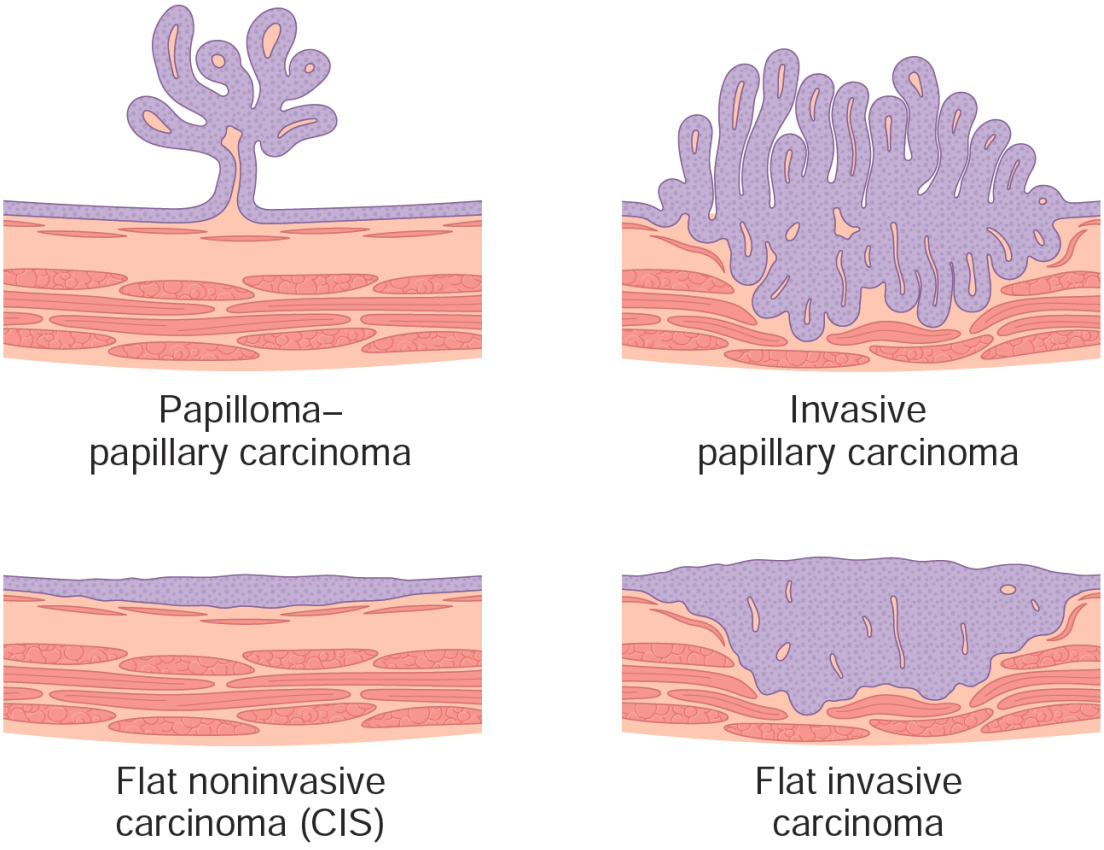
1973 WHO grading system
Urothelial papilloma
Grade 1: Well differentiated
Grade 2: Moderately differentiated
Grade 3: Poorly differentiated
2016 WHO grading system [Papillary lesions]
Low Grade papillary urothelial carcinoma
High Grade papillary urothelial carcinoma
Urothelial papilloma (completely benign lesion)
Papillary urothelial proliferation of low malignant potential (PUNLMP)
2016 WHO grading system [Flat lesions]
Urothelial Carcinoma in situ (always high grade)
Urothelial proliferation of uncertain malignant potential
Reactive atypia (flat lesion with atypia)
Atypia of unknown significance
Urothelial dysplasia

WHO: World health organisation

Urothelial tumours can be classified according to papillary and flat lesions. The risk of invasive disease with low grade papillary cancers is low, with high grade cancers accounting for 95% of invasive disease (50). Papillary urothelial proliferation of low malignant potential (PUNLMP) is nearly exclusively not

invasive with a negligible risk of progression although it can recur (51). Urothelial proliferation of uncertain malignant potential represents a thickened urothelium which is devoid of papillary fronds, and has unknown clinical significance (51). It is occasionally identified at urothelium adjacent to a papillary lesion or in patients with a previous bladder cancer history and may be a precursor to low grade papillary lesion (52). Carcinoma *in situ* represents a form of high grade UCC which can present in isolation or concurrent with papillary disease. Urothelial dysplasia is believed to be preneoplastic with suspicious cytological and morphological changes but short of CIS. It is poorly studied with a high interobserver variability when making a diagnosis. While it is debatable how urothelial dysplasia should be managed, progression in up to 19% of cases have been reported highlighting the requirement for follow-up (53).

Figure 1.3: Schematic diagram of flat and papillary bladder tumours. Adapted from Robbins and Cotran Pathologic Basis of Disease. 9th edition (54)



1.2.2.2 Staging of bladder cancer

Disease stage is determined by depth of invasion of bladder cancer. Tumours are classified to non-muscle invasive bladder cancer (NMIBC) [CIS, pTa, pT1] and muscle invasive bladder cancer (MIBC) [pT2, pT3, pT4] (Figure 1.4). NMIBC accounts for approximately 75% of all bladder cancer diagnosed and MIBC is diagnosed in the remaining 25% (55). Detailed 2002 TNM classification by the Union Internationale Contre le Cancer which was updated in 2009 is shown in Table 1.3 (56).

Figure 1.4: Schematic diagram of pathological stages of bladder cancer. Adapted from Cancer Research UK (43)

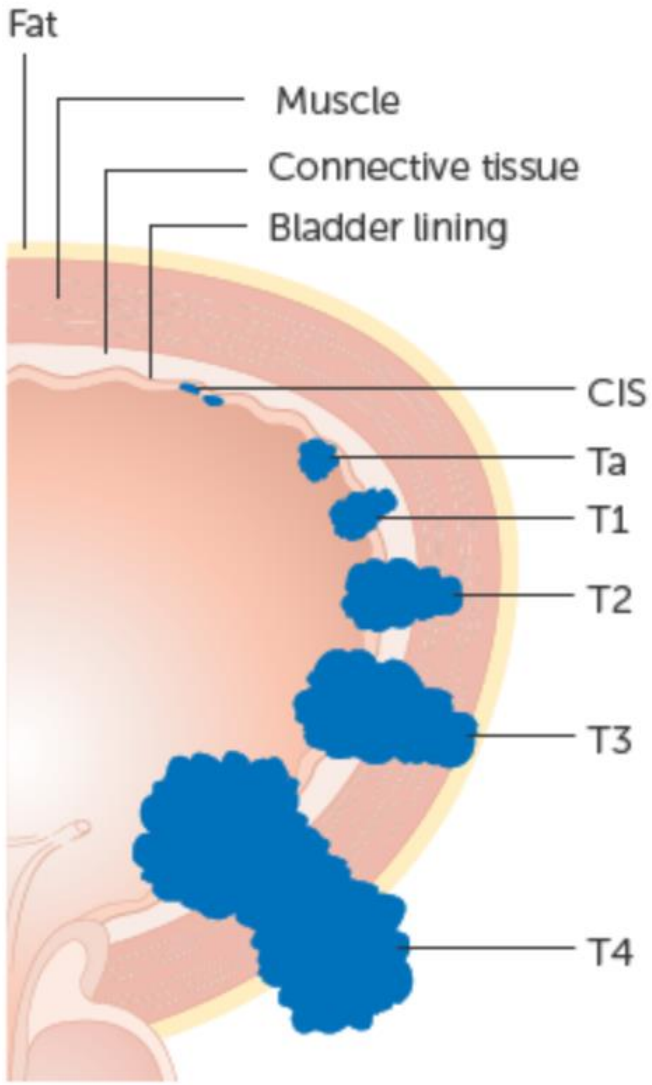


Table 1.3: TNM classification of bladder cancer

T: Primary tumour	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma in situ: “flat tumour”
T1	Tumour invades subepithelial connective tissue*
T2	Tumour invades muscle
	T2a Tumour invades superficial muscle (inner half)
	T2b Tumour invades deep muscle (outer half)
T3	Tumour invades perivesical tissue
	T3a Microscopically
	T3b Macroscopically (extravesical mass)
T4	Tumour invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall
	T4a Tumour invades prostate, uterus, or vagina
	T4b Tumour invades pelvic wall or abdominal wall
N: Lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N2	Metastasis in multiple lymph nodes in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N3	Metastasis in common iliac lymph node(s)
M: Distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

1.2.2.3 Risk classification of bladder cancer

Risk stratification of bladder cancer is used to determine which patients are at risk for disease recurrence and progression, and to determine if adjuvant intravesical treatment or more intensive surveillance is required. Clinical-pathological features such as stage, tumour size and multiplicity are used to determine disease risk. The most commonly used risk classification for disease recurrence and progression is the European Organization for Research and Treatment of Cancer (EORTC) risk table derived from 2,596 patients pooled from seven trials (57). The other NMIBC scoring model is the Club Urológico Español de Tratamiento Oncológico (CUETO) model which is based on 1,062 patients

from four trials (58). Comparison of these two nomograms to an independent cohort of 4,689 patients suggest that both models overestimate the risk of disease progression and recurrence especially in high risk patients (59). The low numbers of patients treated with intravesical BCG would explain this finding. A new EORTC nomogram derived from 1,812 patients treated with 1-3 years of BCG maintenance has recently been published (60). Although this updated risk table reflects current practice and included patients who received maintenance BCG, a limitation of this risk tables is the fact that patients with CIS were not included and no high risk patients underwent repeat transurethral resection. In addition, low risk patients were treated with intravesical BCG which is not recommended in current clinical practice. Hence, this risk tables may underestimate recurrence and progression in low risk NMIBC but overestimate recurrence and progression in high risk disease. In addition, there was no distinction made between patients receiving one or three year BCG maintenance. Table 1.4 compares key predictors for recurrence, progression, cancer specific survival (CSS) and overall survival (OS) for each nomogram.

Table 1.4: Variables predicting early and late recurrence, progression, cancer specific survival and overall survival. Adapted from Tan et al. (61)

	EORTC, 2006 (57)	CUETO, 2009 (58)	EORTC, 2016 (60)
Early Recurrence	-Number of tumours: 2-7, ≥8 -Prior recurrence rate: ≤1/yr, >1/yr -Tumour size: ≥3 cm -Grade: G2, G3 -Presence of CIS -T Category: T1	-Tumour status: recurrent -Gender: Female -Grade: G2, G3 -Tumour size: ≥3 cm -Presence of CIS -Age: 60-70, >70 yr	-Prior recurrence rate: ≤1/yr, >1/yr -Number of tumours: ≥4 -Grade: G2–G3
Late recurrence			-Prior recurrence rate: ≤1/yr, >1/yr -Number of tumours: ≥4
Progression	- Presence of CIS -Grade: G3 -T Category: T1 -Number of tumours: 2-7, ≥8 -Tumour size: ≥3 cm -Prior recurrence rate: ≤1/yr, >1/yr	-Grade: G2, G3 -Age: >70 yr -Tumour status: recurrent -T Category: T1 -Presence of CIS	-Stage: T1 -Grade: G2, G3
Cancer specific survival			-Stage: T1 -Grade: G2, G3
Overall survival			-Increasing age (continuous) -Grade: G2, G3

Yr: year; CIS: carcinoma *in situ*

Risk groups adopted by the UK and European guidelines are based on the old EORTC tables (Table 1.5). The key difference between EAU and NICE is that EAU categorises patients with multiple or recurrent low grade >3cm Ta tumours as high risk disease while it would be under intermediate risk disease according to NICE. NICE has also acknowledge that aggressive variants such as micropapillary tumours are high risk regardless of disease stage as this is reflected in their poor prognosis (62). I have previously published a comparison of international guidelines for NMIBC in the journal *Cancer Treatment Reviews* (61).

Table 1.5: Risk groups of non-muscle invasive bladder cancer stratified by EAU and NICE guidelines. Adapted from Tan et al. (61)

Risk groups	EAU, 2017 (25)	NICE, 2015 (2)
Low	-New solitary pTa low grade (G1/2) <3cm	-Solitary pT1 low grade (G1/2) <3cm -Papillary urothelial neoplasm of low malignant potential
Intermediate	-All others	-Solitary pTa low grade (G1/2) > 3cm -Multifocal pTa low grade (G1/2) -pTa high grade (G2) -Any pTaG2 (unspecified) -Any low risk with recurrence <12 months
High	-Any ≥pT1 -pTa high grade (G3) -p*CIS* -Multiple/ recurrent & >3cm Ta low grade (G1/2)	-Any ≥pT1 -pTa high grade (G3) -p*CIS* -Aggressive variants- nested/ micropapillary

CIS: carcinoma *in situ*

1.2.2.4 Bladder cancer subtypes and treatment options

1.2.2.4.1 Pure urothelial cell carcinoma

Approximately 90% of bladder cancers are UCC. NMIBC is treated by endoscopic resection followed by risk based adjuvant intravesical instillation of chemotherapy or Bacillus Calmette-Guerin (BCG) to reduce the risk of disease recurrence in intermediate and high risk disease respectively (61). Mitomycin C (MMC) represents the most common intravesical chemotherapy used. In the UK, the current recommendation is 6 weekly instillation of Mitomycin C (MMC) for intermediate risk disease and further maintenance MMC is not recommended (63, 64). This is similar to the protocol of the MMC arm in the Southwest Oncology Group (SWOG) 8795 protocol (65).

In contrast, induction and maintenance BCG has been shown to be highly effective and strongly recommended for high risk disease (61). The SWOG regime of 6 weekly induction followed by 3 once weekly on month 3, 6 followed by every 6 months for 3 years is the most common protocol used (66). Maintenance BCG treated patients had a significantly higher recurrence free survival (RFS) compared to induction only (maintenance: 76.8 months vs no maintenance: 35.7 months, $p<0.001$), with an absolute 5 year survival advantage of 5% (maintenance: 83% vs no maintenance: 78%, $p=0.08$) confirming its superiority (66). Two further meta-analysis have reported a reduction in risk of disease progression in maintenance BCG treated patients (67, 68).

In patients with MIBC, cisplatin based neoadjuvant chemotherapy is recommended followed by radical cystectomy. The use of neoadjuvant chemotherapy is supported by level one evidence reporting a 5% absolute survival advantage at 5 years (69). A 28.6% complete response at cystectomy following neoadjuvant chemotherapy has been reported, with a relative risk of overall survival (OS) of 0.45 (95% CI 0.36-0.56, $p<0.001$) (70). Radical radiotherapy is an option for patients not suitable for radical cystectomy although comparable outcomes to cystectomy have been reported in well selected patients (71, 72).

1.2.2.4.2 Urothelial cell carcinoma with divergent differentiation

Up to 33% of patients with UCC have divergent differentiation in which UCC represents the predominant cell type with interspacing cells with other morphological features (73). Table 1.6 describes the different classification of

divergent urothelial carcinoma. Mixed UCC subtypes appear to confer differing survival rates and have been shown to be associated with aggressive disease with a high risk of recurrence even in NMIBC cases and may benefit from early cystectomy. Morphologically, divergent differentiated UCC may show features of non-UCC. Squamous differentiated UCC is defined as the presence of keratinisation or the presence of intercellular bridges while UCC with extravasated mucin with or without signet ring cell have features of adenocarcinoma. Micropapillary UCC is diagnosed by overexpression of HER2 on immunohistochemistry (74).

Generally, UCC with divergent differentiation should be treated according to recommendations of pure UCC (75). A retrospective single centre report of divergent differentiated UCC treated with bladder preservation therapy, which consist of a maximal TURBT followed by chemoradiation for MIBC, suggest that 10-year disease specific survival are similar to pure UCC patients (76). Nevertheless, analysis of cancer registry data suggests that although micropapillary and sarcomatoid UCC were less likely to have non-organ confined disease when treated with neo-adjuvant chemotherapy, this did not translate to a better overall survival potentially due to aggressive tumour biology (77). Patients with neuroendocrine differentiation did have a survival benefit for neoadjuvant chemotherapy and this should be strongly recommended (77).

High risk NMIBC patients with divergent differentiation such as those with sarcomatoid, plasmacytoid and micropapillary features may benefit from early cystectomy due to the high risk of upstaging and disease progression (75). BCG may not be effective in patients with micropapillary variant with a 67% risk of disease progression, including 22% who developed metastatic disease (78). Due

to the rarity of divergent histology UCC and interobserver variability, reports on this subgroup of patients comprise predominantly of retrospective series.

Table 1.6: 2016 WHO classification of divergent urothelial carcinoma

2016 WHO classification of urothelial carcinoma
Invasive urothelial tumours
Infiltrating urothelial carcinoma with divergent differentiation
Nested, including large nested
Microcystic
Micropapillary
Lymphoepithelioma-like
Plasmacytoid/signet ring cell/diffuse
Sarcomatoid
Giant cell
Poorly differentiated
Lipid rich
Clear cell
Tumours of maüllerian type
Tumours arising in a bladder diverticulum

1.2.2.4.3 Non-urothelial cell carcinoma of the bladder

Non-UCC bladder cancer accounts for 10% of bladder cancers which are predominantly adenocarcinoma and squamous cell carcinoma (SCC) (75). Adenocarcinoma of the bladder can be classified to urachal and nonurachal in origin. Patients with urachal adenocarcinoma are younger and have a higher cancer specific survival although more likely to present with metastatic disease as tumour growth can track along the urachus within the detrusor (79). Where localised disease is diagnosed, partial cystectomy with the excision of umbilical stalk is recommended. Meta-analysis suggest that 5-fluorouracil (5-FU) based chemotherapy is more effective than traditional cisplatin based chemotherapy

used for muscle invasive UCC and is a treatment option following disease recurrence or metastatic disease (80).

Radical cystectomy remains the recommended treatment option for non-urachal adenocarcinoma. Early cystectomy is advocated for non-muscle invasive adenocarcinoma with a small case series reporting 100% disease free survival at 5 years (79). Chemotherapy has not been shown to be effective but adjuvant radiation therapy may improve local control (96% vs 53%) (81). These patients should also have a colonoscopy to rule out colorectal pathology.

Pure SCC of the bladder can be classified to bilharzial and nonbilharzial forms with bilharzial disease due to schistosomiasis infection. The main stay of treatment for both forms of SCC is radical cystectomy. Population based studies suggest that SCC presents with more advanced disease compared to UCC (72% vs 52%) (82). Perioperative or postoperative radiotherapy may be advantageous in improving local recurrence although evidence remains limited (83). Systemic chemotherapy has not been effective in improving disease free survival (84).

1.2.3 Non-muscle invasive bladder cancer surveillance

Surveillance of NMIBC using a combination of cystoscopy, upper tract imaging and urinary test is recommended due to the risk of recurrence and progression (85). Up to 52% of NMIBC patients develop recurrence and 20% progress to muscle invasive disease within 5 years (86). The interval between surveillance cystoscopy is dependent on the risk of recurrence (87). The difference in surveillance strategies between guidelines are described in Table 1.7. The three month cystoscopy is essential as it has prognostic implications for tumour

progression and should be performed in all patients regardless of stage and grade of disease (85, 88).

In low risk patients, EAU recommends that patients who are recurrence free at 5 years can be discharged. This is based on a cohort study of 115 low risk patients followed up for a mean duration of 19.4 years where 98% of patients who did not develop recurrence after 5 years remained recurrence free at 20 years (89). National Comprehensive Cancer Network (NCCN), ICUD and Japan Urology Association (JUA) do not specify how frequent surveillance cystoscopy should be performed. The updated NICE guidelines advocate discharging low risk patients after 1 year of no recurrence.

There is limited evidence that intensive cystoscopic surveillance in low risk NMIBC improves overall survival. In addition, cystoscopy is not without morbidity with up to 5.5% of patients developing a urinary tract infection and a long surveillance protocol has significant healthcare cost implications (90, 91). There is increasing evidence that low grade NMIBC infrequently progress and some have even suggested watchful waiting for small recurrent low grade pTa tumours where tumours were only resected when a change in tumour morphology or size was observed (92). A cohort study reported that patients who developed disease progression were predominantly within their first year from initial transurethral resection of bladder tumour (TURBT) supporting NICE recommendations (89).

A retrospective analysis of 152 low grade pTa tumours with a mean follow up of 76 months reported that patients who remain tumour free after 12 months of follow up had a 43% risk of recurrence and 2.6% of patients had stage progression (93). However, this study did not clarify if these low grade cancers were newly diagnosed cancers or recurrence, the latter would be classified as

intermediate risk which will require at least 5 year follow-up. A national survey in the UK suggests that hospitals who adhered to NICE guidelines for low risk cancers have not reported adverse outcomes with no reported cases of stage or grade progression (94). There is no evidence to support the use of upper tract surveillance imaging for low risk NMIBC patients. This is based on patient registry data of 99,338 bladder cancer patients reporting that only 0.7% of low grade bladder cancer patients developed upper tract cancers at a median follow up of 33 months (95).

In high risk NMIBC, yearly cystoscopy is recommended even beyond 5 years. Despite maintenance BCG, disease progression occurred in 19.8% of patients with a cancer specific survival of 88.7% at 5 years (96). The progression rate for high risk NMIBC beyond 5 years is 31% necessitating long term cystoscopy (97). Urinary cytology may be of significant value especially in the surveillance setting of high risk disease due to its high sensitivity (98). Bladder mapping/ PDD cystoscopy, prostatic urethra biopsy, ureteroscopy and CTU should be considered when urinary cytology is positive in the absence of NMIBC recurrence (99). The need for upper tract surveillance is recommended every 1-2 years indefinitely. A retrospective analysis of 193 high risk NMIBC patients treated with BCG with a median follow-up of 86 months reports that high risk patients have an 11 fold odds ratio of developing upper tract cancer compared to low risk NMIBC, emphasising the need for continuous intermittent upper tract surveillance in high risk NMIBC (100).

Table 1.7: Comparison of surveillance protocol of EAU, NCCN, NICE, ICUD and JUA guidelines. Adapted from Tan et al. (61)

	EAU, 2017 (25)	**NCCN, 2018 (101)**	**NICE, 2015 (102)**	**ICUD, 2012 (32)**	**JUA, 2010 (103)**
Low	3-month cystoscopy, then at 12 months and yearly for 5 years then discharge	Cystoscopy at 3 and 12 months then annually for 5 years. As clinically indicated beyond 5 years	Cystoscopy at 3 and 12 months, then discharge Urine cytology/ biomarkers not recommended	Periodic cystoscopy No upper tract imaging	3-month follow up then risk adapted
Intermediate	Adapted to personal/ subjective factors Investigate with cystoscopy and urine cytology	Cystoscopy and urine cytology at 3, 6 and 12 months then every 6 months for year 2 and annually for year 3-5. As clinically indicated beyond 5 years.	Cystoscopy at 3, 9, 18, 30 months then yearly for 5 years in total then discharge Role of urine cytology/ biomarkers not specified		
High	3 monthly cystoscopy & urine cytology for 2 years, then 6 monthly for 5 years and then yearly indefinitely Yearly upper tract imaging with IVU/CTU	Cystoscopy & urine cytology every 3 months for 2 years then every 6 months for years 3-5. Annually from year 5-10. As clinically indicated beyond 10 years. Consider upper tract imaging every 1-2 years Urinary markers optional	Cystoscopy 3 monthly for 2 years then 6 monthly for 2 years then yearly indefinitely Role of urine cytology/ biomarkers not specified	Cystoscopy & urinary cytology 3 monthly for 2 years then 4 monthly for 3rd year then 6 monthly for 4th & 5th year then yearly. If no recurrence, imaging of upper tracts by renal tract ultrasound/ IVU, CTU periodically	

CTU: CT urogram, EAU: European Association of Urology, ICUD: International Consultation on Bladder Cancer, JUA: Japan Urology Association, NCCN: National Comprehensive Cancer Network, NICE: National Institute for Health and Care Excellence, IVU: intravenous urogram

1.3 Renal cancer

1.3.1 Epidemiology

Renal cancer is the 11th most common disease in the world with an estimated 338,000 new cases diagnosed per year worldwide (33). It is estimated that 12,500 new renal cancer cases were diagnosed in the UK in 2015 (104). Majority of renal cancers identified are incidental findings following abdominal imaging for unrelated reasons. The classic trial of flank pain, VH and a palpable abdominal mass is now rare. Small renal masses (tumours ≤4 cm) account for most of the increase in renal cancer cases diagnosed over the last 20 years (105). However, an estimated 20-30% of patients are diagnosed with metastatic disease (106). In fact, the overall mortality following renal cancer has increased between 1983 to 2002 particularly among lesions >7 cm despite the fact that more small renal masses are being diagnosed (105).

Risk factors reported herein relates to renal cell carcinoma (RCC). Environmental risk factors for renal cancer include cigarette smoking and obesity. A meta-analysis suggest that smokers have a 31% higher risk of developing renal cancer compared to non-smokers (107). Cessation of smoking results in a linear decrease in renal cancer risk although this is only apparent following 20 years of smoking cessation (108). Obesity increases the relative risk of renal cancer by 42% based on meta-analysis data (109).

Patient factors include hypertension, genetic and familial syndromes. Population based studies suggest that hypertension was associated with a 2.5 relative risk of developing renal cancer (110). This was independent of sex and this increased risk of developing renal cancer was not evident in patients taking antihypertensive

medications (110). Numerous genetic alterations have been linked with the development of renal cancer. Mutations or epigenetic alteration of the VHL tumour suppressor gene are identified in 80% of renal cancers (111). Hereditary syndromic forms of renal cancer include von Hippel-Lindau syndrome (VHL 3p25–26), hereditary papillary renal cell carcinoma (MET 7q31–34), Birt-Hogg-Dubé syndrome (FLCN 17p11) and tuberous sclerosis (TSC1 9q34 or TSC2 16p13) (112).

1.3.2 Classification of renal cancer

Renal cancer consists of multiple subtypes. Clear cell RCC is the most common subtype, accounting for 80% of cases(113). This is followed by papillary RCC (15%) and chromophobe RCC (5%) (113). Other rarer subtypes include collecting duct RCC and medullary RCC amongst the >24 different subtypes according to the 2013 renal tumour classification (Table 1.8) (113, 114).

Table 1.8 WHO classification of tumours of the kidney (114)

Renal cell tumours Clear cell renal cell carcinoma Multilocular clear cell renal cell carcinoma Papillary renal cell carcinoma Chromophobe renal cell carcinoma Carcinoma of the collecting ducts of Bellini Renal medullary carcinoma Xp11 translocation carcinomas Carcinoma associated with neuroblastoma Mucinous tubular and spindle cell carcinoma Renal cell carcinoma, unclassified Papillary adenoma Oncocytoma **Metanephric tumours** Metanephric adenoma Metanephric adenofibroma Metanephric stromal tumour **Nephroblastic tumours** Nephrogenic rests Nephroblastoma Cystic partially differentiated nephroblastoma	**Mixed mesenchymal and epithelial tumours** Cystic nephroma Mixed epithelial and stromal tumour Synovial sarcoma **Neuroendocrine tumours** Carcinoid Neuroendocrine carcinoma Primitive neuroectodermal tumour Neuroblastoma Phaeochromocytoma **Haematopoietic and lymphoid tumours** Lymphoma Leukaemia Plasmacytoma **Germ cell tumours** Teratoma Choriocarcinoma **Metastatic tumours**

Mesenchymal tumours Occurring Mainly in Children Clear cell sarcoma Rhabdoid tumour Congenital mesoblastic nephroma Ossifying renal tumour of infants Occurring Mainly in Adults Leiomyosarcoma (including renal vein) Angiosarcoma Rhabdomyosarcoma Malignant fibrous histiocytoma Haemangiopericytoma Osteosarcoma Angiomyolipoma Epithelioid angiomyolipoma Leiomyoma Haemangioma Lymphangioma Juxtaglomerular cell tumour Renomedullary interstitial cell tumour Schwannoma Solitary fibrous tumour	

1.3.2.1 Grading of renal cancer

Over the last 30 years, numerous grading systems have been developed for renal cancer. The Fuhrman system is the most commonly used grading system but it is not recommended for chromophobe RCC. The Fuhrman system represents a 4 grade scoring system which is determined based on nuclear diameter, nuclear shape and appearance of nucleoli (Table 1.9) (115). UTUC follows the grading classification as bladder cancer as described in Chapter 1.2.2.1.

Table 1.9 Fuhrman grading system (1982) (115).

Grade	Nuclear diameter	Nuclear shape	Nucleoli
1	Small (~10 µm)	Round, uniform	Absent, inconspicuous
2	Larger (~15 µm)	Irregularities in outline	Visible at x400
3	Even larger (~20 µm)	Obvious irregular outline	Prominent at x400
4	Grade 3 plus bizarre multilobed nuclei ± spindle cell		

The WHO/ International Society of Urological Pathology (ISUP) grading system is currently the recommended scoring system by the WHO (Table 1.10). It has been validated in clear cell, papillary and chromophobe RCC. Similar to the

Fuhrman classification, is it also a 4 grade scoring system. Grade 1-3 tumours are defined based on nucleolar prominence while grade 4 is determined by the presence of pronounced nuclear pleomorphism, tumour giant cells, and/ or rhabdoid and/ or sarcomatoid differentiation (116).

Table 1.10 WHO/ ISUP grading system (116).

Grade	Description
1	Tumour cell nucleoli invisible or small and basophilic at 400 x magnification
2	Tumour cell nucleoli conspicuous at 400 x magnification but inconspicuous at 100 x magnification
3	Tumour cell nucleoli eosinophilic and clearly visible at 100 x magnification
4	Tumours showing extreme nuclear pleomorphism and/or containing tumour giant cells and/or the presence of any proportion of tumor showing sarcomatoid and/or rhabdoid dedifferentiation

1.3.2.2 Staging of renal cancer

The most recent TNM classification update was in 2010. Renal cancer is staged based on the size of the renal mass, extension of tumour into renal vein or vena cave and the presence of tumour invading into Gerota's fascia (117). Full TNM classification for renal cancer is shown in Table 1.11.

Table 1.11 TNM classification of renal cancer

T: Primary tumour	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1a	Tumour ≤4 cm in greatest dimension, limited to kidney
T1b	Tumour >4 cm but not >7 cm in greatest dimension, limited to kidney
T2a	Tumour >7 cm but ≤10 cm in greatest dimension, limited to kidney
T2b	Tumour >10 cm, limited to kidney
T3a	Tumour extending into renal vein or its segmental branches or tumour invading perineal or renal sinus fat but not beyond Gerota fascia
T3b	Tumour grossly extends into the vena cava but below the diaphragm
T3c	Tumour grossly extends into the vena cava but above the diaphragm
T4	Tumour invading beyond the Gerota's fascia (including contiguous extension into the ipsilateral adrenal gland)
N: Lymph nodes	
NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in regional lymph node
M: Distant metastasis	
MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

1.3.3 Treatment of renal cancer

The primary management of localised renal cancer is surgical excision. However, the most recent NCCN guidelines now support the role of active surveillance in small renal mass <2 cm due to a low risk of metastatic potential as well as a 22% likelihood that these small renal mass are benign (118-120). This also is an option for elderly patients who are comorbid where surgery may not be an option.

Surgical excision can be performed either by an open or laparoscopic approach with increasing preference for laparoscopic where technically feasible due to a shorter hospital length of stay and lower analgesia requirement with a comparable oncological outcome (121). For small renal masses (≤4 cm), partial nephrectomy is recommended where technically feasible although this can be also performed in larger tumours, especially exophytic lesions. The rational for

this recommendation is based on the hypothesis that that removing the small renal mass with an adequate margin preserves the remaining renal parenchyma which in turns reduces the risk of cardiovascular disease attributed to chronic kidney disease. Nevertheless, this has not been proven in a randomised controlled trial as well as recently published retrospective population analysis which suggest that long term overall survival for patients treated with either partial nephrectomy and radical nephrectomy were comparable (122, 123). Alternative options to surgical excision include cryotherapy or radiofrequency ablation either via a laparoscopic or percutaneous approach (124). Patients with T1 disease have an estimated 5 year overall survival of 85-90% while for patients with T2 disease, this falls to 67-83% (125).

For T3-T4 renal cancer, tumour thrombus is evident in the renal vein or vena cava hence, patients are treated with nephrectomy with thrombectomy. Vascular control for such cases is essential and hepatic mobilisation or cardiopulmonary bypass may be necessary. Perioperatively, an inferior vena cava filter may be required to reduce the risk of pulmonary embolus. Five year overall survival for T3 and T4 renal cancer ranges from 67-77% and 3-51% respectively (125).

In patients with metastatic renal cancer who are fit, palliative cytoreductive nephrectomy with immunotherapy was historically advocated based on randomised data (126). However, in the era of tyrosine-kinase inhibitors, patients treated with cytoreductive nephrectomy with sunitinib had a comparable overall survival comparable to sunitinib alone (127). Cytoreductive nephrectomy is now no longer advocated in high risk disease although some may argue that low risk metastatic RCC patients may still derive a benefit from cytoreductive nephrectomy (128). Treatment for advanced or metastatic renal cancer is rapidly

evolving (129, 130). A plethora of new drugs particularly immunotherapy in a form of check point inhibitors has been shown to be superior to targeted therapies and cytoreductive nephrectomy may have a role in patients treated with novel immunotherapy drugs although this remains unproven.

1.4 Urinary biomarkers

1.4.1 Commercially available biomarkers

Currently there are six US Food and Drug Administration (FDA) commercially approved urinary tests for clinical use. These are: BTA stat (Polymedco), BTA TRAK (Polymedco), nuclear matrix protein 22 (NMP22) (Matritech), NMP22 BladderCheck Test (Alere), ImmunoCyt (Scimedx) and UroVysion Bladder Cancer Kit (Abbott Molecular). They have an overall sensitivity of between 57-82% and specificity of between 74-88% (131). Table 1.12 summaries the diagnostic ability of these urinary biomarkers and stratifies them according to whether they were used for the evaluation of signs and symptoms suggestive of bladder cancer or NMIBC surveillance. Urinary biomarkers performed better when evaluating patients for a primary diagnosis of bladder cancer than for the monitoring for recurrence. The only exception was quantitative NMP22 and ImmunoCyt where the sensitivity was higher for the detection of recurrence. It has been hypothesised that a 'field effect' exists in bladder cancer, where histologically normal urothelium surrounding the primary tumour may have already acquired somatic (epigenetic or genetic) changes. It is the detection of these changes which may result in a higher false positive rate in the surveillance setting (132). Additionally, the sensitivity of urinary biomarkers are generally higher in high grade and stage cancers.

White light cystoscopy, the gold standard for detection of bladder cancer, has a sensitivity of ≥98% (133). Hence, none of the currently available assays are licensed to be used without cystoscopy as reported sensitivity are significantly lower than this. An inherent flaw of commercial assays is the reliance on single or small panel of markers for example the UroVysion, a FISH based test, uses

Table 1.12 Test performance of FDA approved urinary biomarkers for the evaluation of symptoms and bladder cancer surveillance. Adapted from Chou et al. 2015 (131).

Assay	Assay details	Evaluation of symptoms				Bladder cancer surveillance	
		Number of studies	Sensitivity, OR (95% CI)	Specificity, OR (95% CI)	Number of studies	Sensitivity, OR (95% CI)	Specificity, OR (95% CI)
Quantitative NMP22	Qualitative immunoassay Point of care test. 4 drops of urine.	9	0.67 (0.55 to 0.77)	0.84 (0.75 to 0.90)	10	0.61 (0.49 to 0.71)	0.71 (0.60 to 0.81)
Qualitative NMP22		2	0.47 (0.33 to 0.61)	0.93 (0.81 to 0.97)	2	0.70 (0.40 to 0.89)	0.83 (0.75 to 0.89)
Qualitative BTA	Agglutination reaction/ qualitative immunoassay. 2 ml of urine	8	0.76 (0.67 to 0.83)	0.78 (0.66 to 0.87)	11	0.60 (0.55 to 0.65)	0.76 (0.69 to 0.83)
Quantitative BTA		1	0.76 (0.61 to 0.87)	0.53 (0.38 to 0.68)	2	0.58 (0.46 to 0.69)	0.79 (0.72 to 0.85)
UroVysion	Identify aneuploidy of chromosome 3, 7, 17 and loss of chromosome 9p21by fluorescence in situ hybridisation	2	0.73 (0.50 to 0.88)	0.95 (0.87 to 0.98)	7	0.55 (0.36 to 0.72)	0.80 (0.66 to 0.89)
ImmunoCyt	3 fluorescence antibodies: M344 LDQ10 with fluorescein & 19A211 with Texas red	6	0.85 (0.78 to 0.90)	0.83 (0.77 to 0.87)	7	0.75 (0.64 to 0.83)	0.76 (0.70 to 0.81)
Cxbladder	4 gene mRNA assay: CDC2, HOXA13, MDK, IGFBP5	1	0.82 (0.70 to 0.90)	0.85 (0.81 to 0.88)			

four genomic regions (aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus) and NMP22 detects a single protein. The heterogenous nature of bladder cancer suggest that genomic alterations not interrogated by these assays will be missed and this can have significant consequence for the prognosis and management of patients.

1.4.2 The UroMark assay

Epigenetic alterations such as DNA methylation play a key role in the development of cancer by either silencing tumour suppressor genes or overexpression of oncogenes (134). DNA methylation makes an ideal non-invasive biomarker for the detection and surveillance of cancer because of its ontogenic plasticity and tissue specificity (135). A number of emerging assays based on epigenomic panels have shown the potential utility of DNA methylation changes as urine biomarkers for the detection of bladder cancer (136-138). However, an inherent weakness of reported tests is the limited number of targets which limits the sensitivity of a diagnostic assay.

Next generation DNA sequencing represents the next phase of biomarker discovery. Microdroplet PCR amplification of bisulfite- converted DNA followed by next generation sequencing of targeted loci allows for simultaneous amplification of >4,000 targeted loci (139). The Kelly-Feber laboratory have previously developed a highly multiplexed targeted bisulfite sequencing assay to detect bladder cancer specific epigenetic alterations in urinary sediment (140). This assay utilises a micro-droplet PCR platform (Thunderstorm, RainDance Technologies, Lexington, MA, USA) which allows the analysis of a panel of 150

epigenetically altered loci which can accurately discriminate between bladder tumour and normal urothelium.

The UroMark was developed based on genome- wide DNA methylation profiling of 86 bladder cancers and 30 age- matched control urothelium snap frozen tissue using the Infinium 450k methylation array (Illumina, San Diego, CA, USA). This was subsequently validated using an independent dataset from The Cancer Genome Atlas (TCGA) which comprised of 144 muscle- invasive bladder cancer and 20 normal urothelium. Subsequent validation cohorts comprised of urine samples of two patient cohorts investigated for haematuria which comprised of 86 (52 bladder cancer, 34 non- bladder cancer) and 205 (55 bladder cancer, 133 non-bladder cancer) patients respectively.

The selection of 150 loci was determined based on potential biomarker candidates with no or minimal DNA methylation (β values <10%) in non-cancer urothelium, blood and control urothelium and high DNA methylation levels (β values >50%) in bladder cancer. Potential targets which fulfilled this requirement were incorporated to generate a random forest classifier model. Random forest is a form of machine learning where multiple decision trees and created and are merged together to develop a more accurate and reliable prediction. This was then tested in all validation cohorts and bootstrapped 100 times to develop the final optimised model. The sensitivity and specificity of the 150 target loci in the final independent validation cohort of 78 bladder cancer urine samples and 98 non-cancer controls was 96% and 97% respectively with a ROC area under the curve of 0.96 (140).

1.4 Aims of thesis

The aim of this thesis is to optimise the investigation of haematuria and bladder cancer. Specifically, I intend to:

- Determine the contemporary incidence of urinary tract cancer in patients presenting with haematuria in the UK.
- Determine the diagnostic accuracy of imaging and urinary cytology to detect urinary tract cancers.
- Undertake a systematic review of the urinary biomarkers for the diagnosis of bladder cancer.
- Determine and validate the diagnostic performance of a novel urine biomarker-UroMark, for the detection of bladder cancer in patients presenting with haematuria.
- Develop and externally validate a risk assessment tool to predict which patients will benefit from haematuria investigations.
- Determine patients' acceptance of a urine based biomarker as an alternative test to cystoscopy or in combination with cystoscopy in a patient cohort undergoing surveillance cystoscopy using quantitative analysis and semi-structured qualitative interviews.

CHAPTER 2 : MATERIAL & METHODS

2.1 Studies used for thesis

The work undertaken in this thesis draws on data extracted from two prospective multicentre studies, DETECT I and DETECT II. The aim of the DETECT studies were to validate the results of the UroMark, a urinary biomarker, designed and previously validated by the Kelly-Feber laboratory.

The remit of my thesis extended beyond the primary aims of the clinical studies and the results report in the subsequent chapters were based on secondary outcomes which were incorporated when I amended the overall design of the DETECT I and II protocols. The DETECT study design is set out in this chapter along with the primary objectives and sample size calculations. The study protocol of DETECT I and DETECT II have been previously published in the journal *BMC Cancer* (1). The secondary endpoints which were used for my work are described in detail in the subsequent chapters with sample size calculations and necessary protocol amendments described where applicable. The Standards for Reporting of Diagnostic Accuracy (STARD) guidelines was adhered to ensure diagnostic methodology, results and reporting were reliable.

2.2 Study populations

2.2.1 DETECT I study

DETECT I represents a prospective multicentre observational study recruiting patients with haematuria throughout the UK. All patients were referred by their general practitioner to secondary care for haematuria investigations.

2.2.1.1 Study endpoints

Primary endpoint:

1) Determine the NPV of the UroMark assay in a prospective patient cohort investigated for haematuria

Secondary endpoint which were incorporated as part of the protocol amendment:

1) Determine the incidence of urinary tract cancer in patients presenting with haematuria
2) To compare and assess the reliability of the NICE guidelines to identify patients with urinary tract cancer
3) Determine the diagnostic performance of imaging, cystoscopy and urine cytology for the detection of urinary tract cancers
4) To develop and validate a risk assessment tool to determine which patients would benefit from haematuria investigations following a presentation of haematuria

2.2.1.2 Patient selection

Study inclusion criteria include:

1) Patients ≥18 years of age
2) Patients undergoing cystoscopy for VH or NVH
3) All patients must have upper tract imaging (either RBUS, CT KUB or CTU) within 12 weeks of registration into the study

Study exclusion criteria include:

1) Patients unwilling to have cystoscopy and/ or upper tract imaging

Patients were recruited from 40 hospitals from 36 NHS Trust as shown in Chapter 11.2 Appendix A1.

2.2.1.3 Assessment

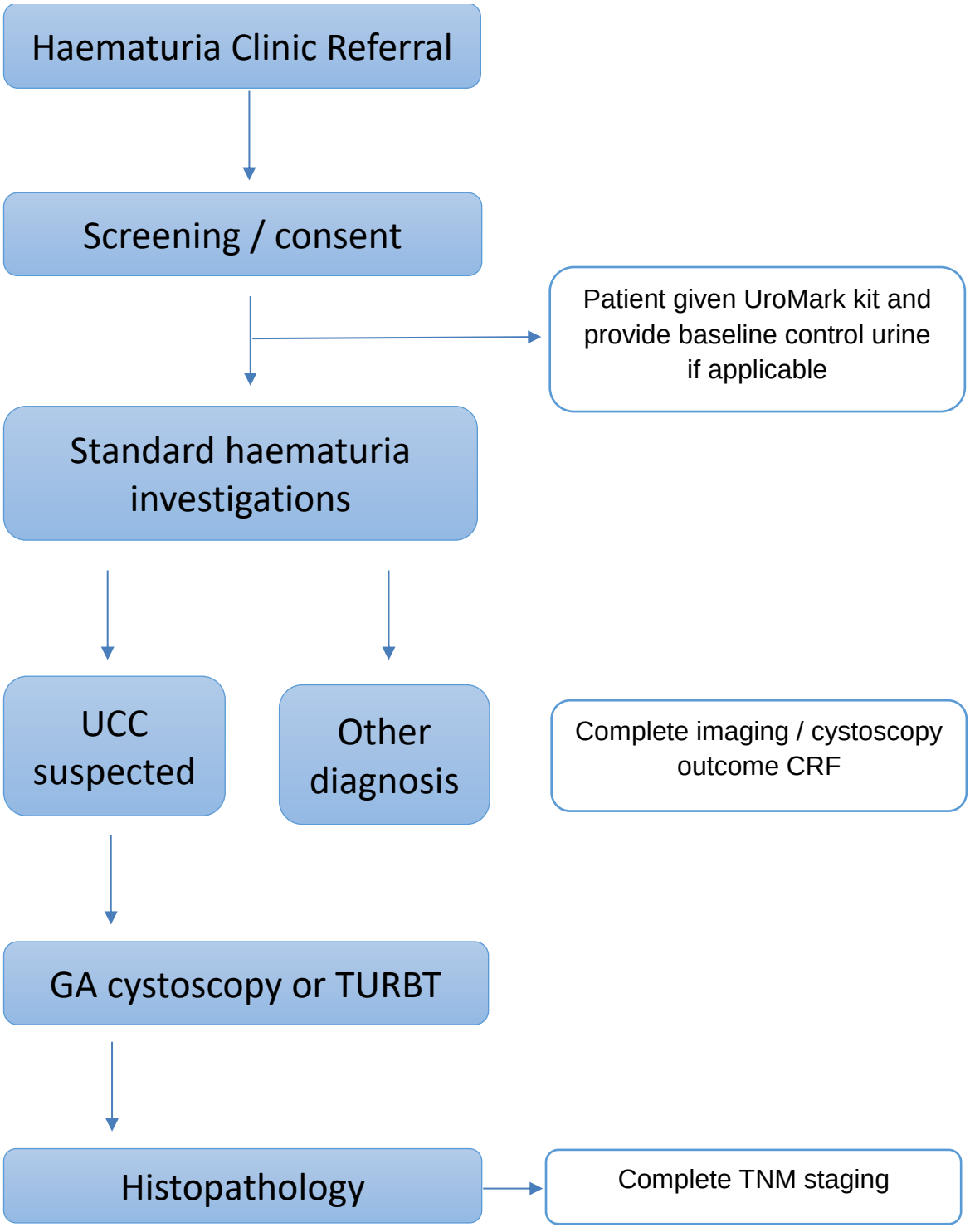
The study schedule for DETECT I is shown in Figure 2.1. Patients were screened for eligibility for inclusion into the study. A patient information sheet (Appendix A4) and written informed consent (Appendix A5) were obtained from each patient before cystoscopy was performed. Clinical evaluation comprised of medical history and examination. The use of any additional urinary biomarkers such as urine cytology was at the discretion of the clinician. Similarly, while upper tract imaging was mandatory, the choice of imaging was determined by the clinician or local hospital policy. Patient demographics including age, gender, occupation, ethnicity and smoking history were recorded using an electronic case record form (eCRF) (Appendix A6). A UroMark urine collection kit was provided to each patient.

Following cystoscopy, patients with a suspected diagnosis of bladder cancer underwent TURBT or cystoscopy with bladder biopsy. The diagnosis of bladder cancer was defined as histopathological confirmation of disease following TURBT or bladder biopsy. Bladder cancer was classified according to TNM WHO tumour classification: pTa, pT1 or ≥pT2 with or without CIS, or isolated CIS and tumour grade as G1, G2 or G3 (73). Risk stratification of bladder cancer was performed based on clinical-pathological features according to the EAU risk classification (25).

The UroMark collection kit was used to collect patients' urine sample at home (Figure 6.1). Urine sampling was performed using the UroMark urine collection

kit anytime ≥48 hours following cystoscopy to negate the effects of instrumentation. Patients labelled the sample tubes with their initials; date of birth and date and time urine sample was collected. Urine sample was collected using a plastic lined urine collection container (MedDX Solutions, Hereford, UK). Patients collected urine at any time and voided directly into the container without the need for a midstream sample. The urine was then transferred into three 25 ml urine collection tubes with a preservative at the base of the tube to reduce the risk of bacterial growth and maintain DNA integrity. Urine can be collected from a single void or multiple voids depending on patients' preference. Following filling, all three urine tubes were placed back into the protective plastic clamshell with an absorbent surface to prevent the spillage of urine. The package was then placed in a prepaid envelope and posted using the Royal Mail to the receiving laboratory at University College London (UCL). Patients with tumour were reminded by telephone call to collect and post a urine sample before undergoing TURBT. Urine samples collected following TURBT or bladder biopsy were excluded for UroMark analysis.

Figure 2.1: Study schedule for DETECT I. Adapted from Tan et al. (1)



UCC: urothelial cell carcinoma of the bladder
GA: General anaesthetic
TURBT: Transurethral resection of bladder tumour

Figure 2.2: UroMark collection kit.

2.2.1.4 Sample size and power calculations

The original sample size calculation was to determine the robustness of the UroMark assay based on an estimated NPV of 98%. Using the exact binomial method which gives a lower bound of a 95% CI of 96.75%, 800 negative test results would be required. Assuming 90% of all patients investigated for haematuria will not have cancer, at least 889 evaluable urine samples will be required. With this sample size, the uncertainty in the estimated NPV will be less than 1% if the NPV is higher than 98%. To ensure with confidence that the final study patient cohort was adequately powered, patients were recruited until at least 89 tumours were identified.

2.2.1.5 Study registration details

The study protocol was approved by Health Research Authority: North West Liverpool Central Research Ethics Committee on the 9th March 2016 (IRAS project ID: 179245, Appendix A2; REC reference: 16/NW/0150, Appendix A3). DETECT I is registered on clinicaltrials.gov NCT02676180.

2.2.2 DETECT II study

DETECT II is a multicentre observational study recruiting patients with cystoscopic confirmation of bladder cancer. All patients had a cystoscopy and a visual diagnosis of bladder cancer.

2.2.2.1 Study endpoints

Primary endpoint:

1) Perform a sensitivity analysis of the UroMark in an enriched patient cohort of bladder cancer

Secondary endpoints:

2) Determine patient's experience of being diagnosed with bladder cancer
3) Determine patient's views and opinion of cystoscopy vs urinary biomarker as part of surveillance for NMIBC

2.2.2.2 Patient selection

Study inclusion criteria include:

1) Patients with a suspicion of new or recurrent bladder cancer following cystoscopy

Study exclusion criteria include:

1) Patients unwilling to have TURBT

Patients were recruited from 52 hospitals from 48 NHS Trusts (Chapter 11.2 Appendix A2).

2.2.2.3 Assessment

2.2.2.3.1 Bladder cancer diagnosis and follow-up protocol

The DETECT II study schedule is shown in Figure 2.2. Patients were screened and included into the trial following cystoscopic suspicion confirmation of a new or recurrent bladder tumour. Each patient was provided with a patient information sheet (Appendix A9) and written consent (Appendix A10) was obtained from eligible patients. Baseline urine samples were collected using the same UroMark urine collection kit. Urine collection was performed prior to TURBT. Histopathological confirmation of cancer was used as the reference standard. Bladder cancer was classified according to tumour stage (isolated CIS, Ta, T1, ≥T2) and grade (G1, G2, G3) as well as the presence of concurrent CIS (73).

All patients received standard of care tests and investigations following a diagnosis of bladder cancer. Following TURBT, a single instillation of intravesical chemotherapy was recommended unless clinically contraindicated. Patients with intermediate risk NMIBC were recommended to have an induction course of six

weekly intravesical chemotherapy instillation. A repeat resection was recommended for pathological stage pT1 tumours to exclude residual detrusor muscle invasion (≥pT2). A 6 weekly induction and maintenance course of intravesical BCG was recommended for patients with high risk NMIBC which is consistent with international consensus (87).

Following TURBT, patients with NMIBC had periodic surveillance cystoscopy every 3 to 12 months depending on disease risk in accordance to local hospital guidelines (Table 3.3). Patients were asked to provide a urine sample using a UroMark urinary collection kit every 3 months for 24 months. Urine samples were matched with clinical findings on cystoscopy. All patient demographic and clinical data were recorded in an eCRF (Appendix A11).

2.2.2.3.2 Patient questionnaire

A purpose-designed questionnaire to assess patients' perspectives on cystoscopy and the use of a urinary test to detect bladder cancer in the surveillance setting was sent by post to patients with NMIBC 6 months following enrolment into DETECT II. The questionnaire was designed to be self-explanatory and capture the following data:

- Education level
- Complications following cystoscopy experienced
- Pain, anxiety and overall experience with cystoscopy
- Standard gamble to assess the threshold of acceptability of a urinary assay
- Opinion on increasing the interval of cystoscopy by adding a urinary assay
- Reasons for patient's choice on urinary assay compared to cystoscopy

Patients also completed the validated Brief Illness Perception Questionnaire, a nine-item scale designed to assess the cognitive and emotional state of patients. The complete questionnaire is shown in Appendix A12.

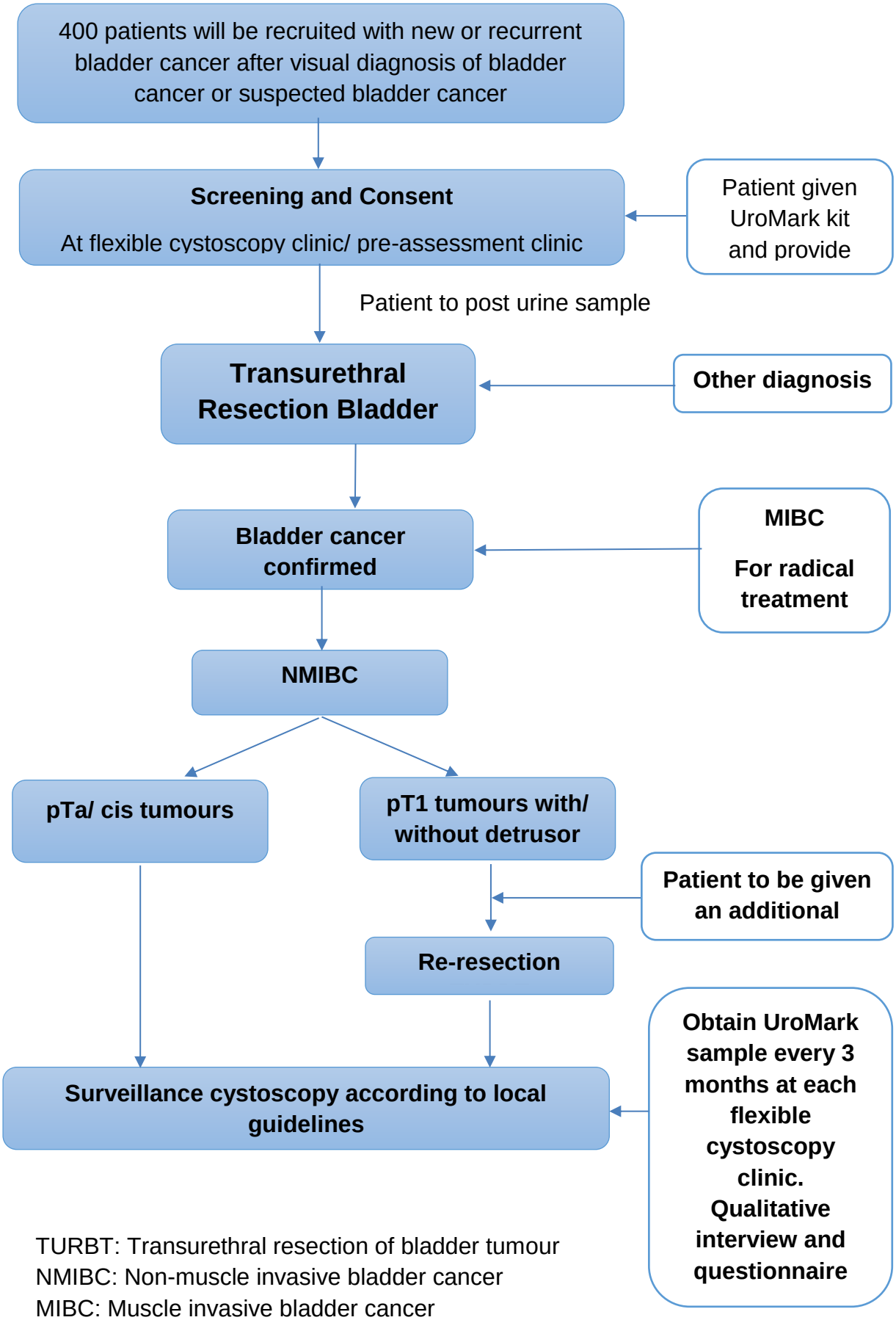
2.2.2.3.3 Qualitative analysis

Selected patients were invited for a telephone interview to further determine their experience of being diagnosed with bladder cancer and having cystoscopy compared to a urinary test as a method of bladder cancer surveillance. Twenty patients participated in a qualitative analysis of semi-structured interviews. Interviews were conducted after 6 months following enrolment to ensure patients have some experience collecting urine samples for biomarker analysis to ensure they have experienced both cystoscopy and urine-based testing.

Table 2.1: Table of Assessments in DETECT II. Adapted from Tan et al. (1).

Visit	**Baseline Before TURBT**	**Month 3**	**Month 6**	**Month 9**	**Month 12**	**Month 15**	**Month 18**	**Month 21**	**Month 24**
Inclusion criteria	x								
Smoking history	x								
Visual Diagnosis of bladder cancer	x								
Surveillance Cystoscopy:									
Low risk		X			X				
Intermediate risk		X		X			X		
High risk		X	X	X	X	X	X	X	X
UroMark Test	x	X	X	X	x	X	X	x	x
Qualitative interview and questionnaire:									
Low risk						X			
Intermediate risk						X			
High risk						X			

Figure 2.3: Study Schedule DETECT II. Adapted from Tan et al. (1)



TURBT: Transurethral resection of bladder tumour
NMIBC: Non-muscle invasive bladder cancer
MIBC: Muscle invasive bladder cancer

2.2.2.4 Sample size and power calculations

The power calculation for the sensitivity analysis was determined based on the assumption of 95% sensitivity for the detection of Grade 1, 2 and 3 bladder cancers with a 95% CI ranging between 92.3% and 97.0%. This will require a minimum of 380 urine samples from bladder cancer patients. To ensure the UroMark assay can detect low grade bladder cancer, we will ensure that the total cohort will comprise of at least 15%–20% of low grade disease. In the event there are less than 60 low grade tumours recruited into DETECT II, we aim to enrich the cohort with cases from other clinical trials where urine samples have been collected from patients with low grade disease with the same methodology. It is estimated that the sensitivity for detection of low grade cancers will be 80% with a 95% CI of between 70.8% to 87.3%.

2.2.2.5 Study registration details

DETECT II study protocol received Health Research Authority: London-Stanmore Research Ethics Committee approval on the 30th August 2016 (IRAS project ID: 203022, Appendix A7; REC reference: 16/LO/1044, Appendix A8). This trial is registered on clinicaltrials.gov NCT02781428.

CHAPTER 3 CHAPTER 3: INCIDENCE OF URINARY TRACT CANCER IN PATIENTS WITH HAEMATURIA

3.1 Introduction

VH is regarded as a ‘red flag’ sign requiring urgent referral for investigation to exclude urinary tract cancer (2). The significance of NVH is less clear and it is estimated that 2.5% of the population will test positive for dipstick haematuria (141). Significant NVH is defined as a urine dipstick RBC score of ≥1+ on ≥2 occasions in the absence of UTI (13). Historic reports from secondary care suggest that up to 20% of patients with VH and 5% of patients with NVH referred for investigation will have a diagnosis of urinary tract cancer (3).

There is a lack of consensus among national guideline bodies relating to which patients would benefit from haematuria investigations (26). In 2015, the NICE recommended that patients aged ≥45 years with VH and ≥60 years with NVH with either dysuria or a raised white cell count on blood test should be referred on a 2-week suspected cancer pathway (2). Non-urgent referral can be considered for patients ≥60 years with recurrent or persistent unexplained UTI with or without haematuria (2). The AUA recommends that all patients presenting with VH and patients with NVH, defined as ≥3 red blood cells per high power field, who are ≥35 years should be investigated (18). In contrast, the National Board of Health and Welfare of Sweden has recommended abandoning referral of cases with NVH for investigation (30).

There is a lack of high quality evidence to inform recommendations for the investigation of haematuria and reliance on expert consensus is necessary but this will have inherent bias (142). Specific age thresholds for VH and NVH adopted in guidelines are based on the fact that patients presenting with VH and older patients are more likely to have a diagnosis of bladder cancer.

In this chapter, I report the incidence of urinary tract cancer diagnosed following referral for investigation of VH or NVH in a contemporary multicentre study. I also compared the different age thresholds used by published haematuria guidelines to determine the appropriateness of age thresholds for referral. Results from this chapter have recently been published in the journal *European Urology* (143).

3.2 Methods

3.2.1 Patient selection

Patients from one-stop haematuria clinics across 40 UK hospitals were recruited between March 2016 and June 2017. Cases were referred from primary care to secondary care following a presentation of VH or NVH after excluding UTI. Referral for investigations were made at the discretion of the primary care physician. VH was defined as the presence of blood in urine witnessed and confirmed by the patient or general practitioner while NVH was defined as urine dipstick of ≥1+ of blood on ≥2 occasions. The inclusion criteria were patients ≥18 years old who were undergoing cystoscopy for VH or NVH and had upper tract imaging within 12 weeks of study registration. All patients were recruited into the study prior to undergoing cystoscopy.

3.2.2 Interventions

Clinical evaluations comprised of medical history and physical examination. Patient demographics including age, gender, occupation, ethnicity and smoking history were recorded. The following occupations were defined as occupational risk factors: gardener, painter, hairdresser/ barber, textile worker or metals factory worker. Urinary tract cancers comprised of bladder cancer or upper tract tumours (renal cancer and UTUC). Cystoscopy was performed and TURBT or biopsy under anaesthesia was performed if there was a suspicion of bladder cancer. The reference standard for bladder cancer was histopathological confirmation of tumour according to the TNM WHO tumour classification (73). EAU risk

classification of bladder cancer was determined according to clinical-pathological features (25).

Upper tract imaging was performed for all cases. Imaging comprised of one of more radiological imaging techniques: RBUS, CTU and/ or CT KUB. DETECT I is a pragmatic observational design study and choice of upper tract imaging and the decision to perform more than one imaging modality was according to local departmental guidelines. The presence of UTUC was confirmed histologically either by ureteroscopic biopsy or following nephroureterectomy. The reference standard for renal cancer was histopathological diagnosis when available or on review of imaging at a multidisciplinary team (MDT) meeting where active surveillance was recommended. CT confirmation of renal colic was used as the reference standard for renal colic when both CT and RBUS were performed.

3.2.3 Statistical analysis

Continuous data such as mean, median, interquartile range and 95% confidence interval were reported using descriptive statistics. Categorical variables were compared using Chi-square test. T-test was used to compare continuous variables. Normal distribution was assumed. Missing data were reported as not known. SPSS v22 (IBM Corp, Armonk, New York, USA) was used for statistical analysis. Statistical significance was set at two-sided p value <0.05.

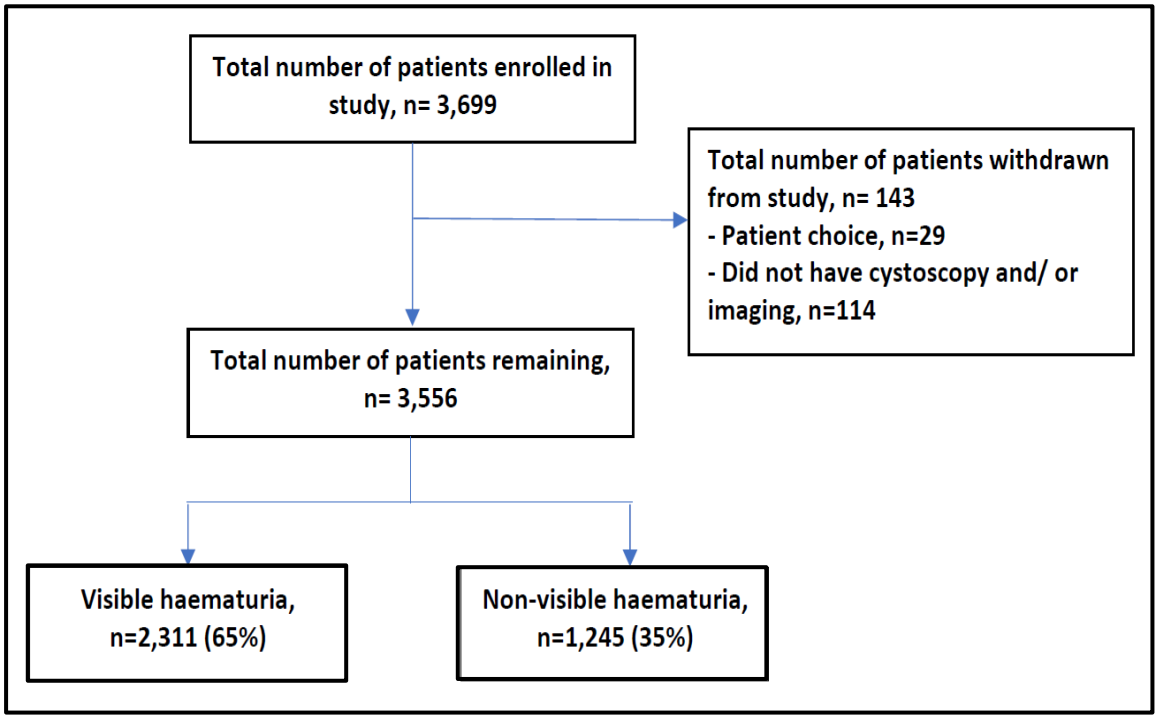
For this secondary endpoint, the sample size was calculated to determine the incidence of upper tract cancers with a high precision. Hence assuming an upper tract cancer incidence of 1% and 7% precision (incidence of 1% with 95% CI of 0.07%), a minimum of 3,105 patients was required (144).

3.3 Results

3.3.1: Patient cohort

3,699 patients were recruited into DETECT I of which 3,556 patients were included in the final analysis (Figure 3.1).

Figure 3.1: Flow diagram of patients recruited into study



3.3.2 Patient demographics

Patient demographics for the entire cohort stratified according to the presence or absence of urinary tract cancer were described in Table 3.1. The overall median age was 67.7 years (IQR: 56.6, 75.8) with male patients (69.8 years (IQR: 58.3, 77.0)) older than female patients (64.4 years (IQR: 54.2, 73.7). At presentation, 2,311 (65.0%) patients had VH, 2,112 (59.4%) patients were male and 1,896 (53.3%) patients had a smoking history. Older patients ($p<0.001$), patients with

VH (p<0.001), male patients (p<0.001), current or previous smoking history (p<0.001), white patients (p=0.021) and retired patients (p<0.001) were significantly associated with a diagnosis of urinary tract cancer. An association between bladder cancer and older patients, male gender, presentation of VH, smoking history, white patients, and retired patients were also observed (Table 3.2).

3.3.3 Diagnosis of patients investigated for haematuria stratified according to type of haematuria and gender

To understand the relationship between a diagnosis of urinary tract cancer, patients were stratified to VH or NVH at presentation and gender (Table 3.3). Urinary tract cancers were identified in 352 (9.9%) patients referred for investigation for haematuria (13.5% of VH cases and 3.1% of NVH cases). The incidence of urinary tract cancer in patients presenting with VH and NVH according to sex were 15.4% and 4.8% for male patients and 9.2% and 2.0% for female patients respectively. Bladder cancer was the most common urinary tract cancer accounting for 81.8% of all malignancies. Upper tract tumour was detected in 56 (1.5%) of all patients, accounting for 15.9% of cancers detected. Of these, 66% were renal cancers and 33.9% were UTUC. Exclusively, all 19 UTUC and 32 (86.5%) renal cancers presented following an episode of VH. Other radiological diagnosis included renal stone disease confirmed in 270 (7.6%) patients. Angiomyolipoma and pelvis ureteric junction obstruction were identified in <1% of patients.

Table 3.1: Patient demographics stratified according to presence or absence of urinary tract cancer

	All patients (n=3,556)	Urinary tract cancer (n=352)	No urinary tract cancer (n=3,204)	p value
Age (median, IQR)	67.7 (57, 76)	74.2 (67, 81)	66.8 (56, 75)	<0.001
Age (mean, range)	65.7 (19-99)	73.0 (28-96)	64.9 (19-99)	
Haematuria, n (%):				<0.001
Visible	2,312 (65.0)	313 (88.9)	1,999 (62.4)	
Non-visible	1,244 (35.0)	39 (11.1)	1,205 (37.6)	
Gender, n (%):				<0.001
Male	2,112 (59.4)	272 (77.3)	1,840 (57.4)	
Female	1,444 (40.6)	80 (22.7)	1,364 (42.6)	
Ethnicity, n (%):				0.023
White	3,080 (86.6)	327 (92.9)	2,753 (85.9)	
South Asian	86 (2.4)	5 (1.4)	81 (2.5)	
Afro-Caribbean	51 (1.4)	2 (0.6)	49 (1.5)	
Oriental	15 (0.4)	0 (0)	15 (0.5)	
Mix	31 (0.9)	2 (0.6)	29 (0.9)	
Other	23 (0.6)	2 (0.6)	21 (0.7)	
Not known	271 (7.6)	14 (3.9)	256 (8.0)	
Smoking history, n (%):				<0.001
Non-smoker	1,528 (43.0)	111 (31.5)	1,417 (44.2)	
Current/ ex-smoker	1,896 (53.3)	232 (65.9)	1,664 (52.0)	
Not known	132 (3.7)	9 (2.6)	123 (3.8)	
Employment status, n (%):				<0.001
Full time/ part time work/ study/ home maker	1,518 (42.7)	84 (23.9)	1,434 (44.8)	
Retired	1,764 (49.6)	248 (70.5)	1,516 (47.3)	
Unemployed	78 (2.2)	4 (1.1)	74 (2.3)	
Disability	40 (1.1)	2 (0.6)	38 (1.2)	
Not known	156 (4.4)	14 (4.0)	142 (4.4)	
Occupational risk factor*, n (%)				0.708
Yes	531 (14.9)	54 (15.4)	477 (14.8)	
No	2,756 (77.5)	274 (77.8)	2,482 (77.5)	
Not known	269 (7.6)	24 (6.8)	246 (7.7)	

Table 3.2: Patient demographics stratified according to bladder cancer diagnosis

	All patients (n=3,556)	Bladder cancer (n=288)	No bladder cancer (n=3,268)	P value
Age (median, IQR)	67.7 (57, 76)	74.3 (67, 81)	66.8 (56, 75)	<0.001
Age (mean, range)	65.7 (19-99)	73.2 (28-96)	64.9 (19-99)	
Haematuria, n (%):				<0.001
Visible	2,311 (65.0)	255 (88.5)	2,057 (62.9)	
Non-visible	1,245 (35.0)	33(11.5)	1,211 (37.1)	
Gender, n (%):				<0.001
Male	2,112 (59.4)	230 (79.9)	1,882 (57.6)	
Female	1,444 (40.6)	58 (20.1)	1,386 (42.4)	
Ethnicity, n (%):				0.122
White	3,080 (86.6)	266 (92.4)	2,814 (86.1)	
South Asian	86 (2.4)	4 (1.4)	82 (2.5)	
Afro-Caribbean	51 (1.4)	2 (0.7)	49 (1.5)	
Oriental	15 (0.4)	0 (0)	15 (0.5)	
Mix	31 (0.9)	2 (0.7)	29 (0.9)	
Other	23 (0.6)	2 (0.7)	21 (0.7)	
Not known	270 (7.6)	12 (4.2)	258 (7.9)	
Smoking history, n (%):				<0.001
Non-smoker	1,528 (43.0)	89 (31.2)	1,439 (44.0)	
Current/ ex-smoker	1,896 (53.3)	190 (65.6)	1,706 (52.2)	
Not known	132 (3.7)	9 (3.2)	123 (3.8)	
Employment status, n (%):				<0.001
Full time/ part time work/ study/ home maker	1,518 (42.7)	59 (20.5)	1,459 (44.6)	
Retired	1,764 (49.6)	211 (73.3)	1,553 (47.5)	
Unemployed	78 (2.2)	3 (1.0)	75 (2.3)	
Disability	40 (1.1)	2 (0.7)	38 (1.2)	
Not known	156 (4.4)	13 (4.5)	143 (4.4)	
Occupational risk factor, n (%)				0.819
Yes	531 (14.9)	44 (15.3)	487 (14.9)	
No	2,756 (77.5)	223 (77.4)	2,533 (77.4)	
Not known	269 (7.6)	21 (7.3)	251 (7.7)	

3.3.4 Incidence of urinary tract cancer according to age

Patients were stratified by gender, type of haematuria at presentation and diagnosis of urinary tract cancers according to age deciles to explore the relationship between age threshold at referral and the detection of urinary tract cancer (Table 3.4A & 3.4B). The incidence of urinary tract cancers was lower in younger patients and in patients with NVH compared to VH (3.1% vs 9.9%). Peak incidence of urinary tract cancer was between 70-89 years. No UTUC was diagnosed in patients presenting with NVH. Urinary tract cancers were rare in patients <40 years with VH; the overall reported incidence was 0.85% (male 1.5%, females 0%). Similarly, no NVH patients <40 years were diagnosed with urinary tract cancer.

Table 3.3: Diagnosis of patients investigated for haematuria stratified according to haematuria type and gender

	All patients			Male			Female		
	Any haematuria (n=3,556)	**VH (n=2312)**	**NVH (n=1244)**	**Any haematuria (n=2,112)**	**VH (n=1608)**	**NVH (n=504)**	**Any haematuria (n=1,444)**	**VH (n=704)**	**NVH (n=740)**
Any urinary tract cancer, n (%)	352 (9.9)	313 (13.5)	39 (3.1)	272 (12.9)	248 (15.4)	24 (4.8)	80 (5.5)	65 (9.2)	15 (2.0)
Bladder cancer, n (%)	288 (8.1)	255 (11.0)	33 (2.7)	230 (10.9)	207 (12.9)	23 (4.6)	58 (4.0)	48 (6.8)	10 (1.4)
Renal cancer, n (%)	37 (1.0)	32 (1.4)	5 (0.4)	23 (1.1)	22 (1.4)	1 (0.2)	14 (1.0)	10 (1.4)	4 (0.5)
Upper tract urothelial cancer, n (%)	19 (0.5)	19 (0.8)	(0)	12 (0.6)	12 (0.7)	0 (0)	7 (0.5)	7 (1.0)	0 (0)
Prostate cancer, n (%)	9 (0.3)	9 (0.4)	0 (0)	9 (0.4)	9 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)
Stone disease, n (%)	270 (7.6)	215 (9.3)	55 (4.4)	185 (8.8)	165 (10.3)	20 (4.0)	85 (5.9)	50 (7.1)	35 (4.7)
Angiomyolipoma, n (%)	17 (0.5)	8 (0.3)	9 (0.7)	4 (0.2)	3 (0.2)	1 (0.2)	13 (0.9)	5 (0.7)	8 (1.1)
Pelvic ureteric junction obstruction, n (%)	8 (0.2)	7 (0.3)	1 (<0.1)	5 (0.2)	5 (0.3)	0 (0)	3 (0.2)	2 (0.3)	1 (0.1)

NVH: non-visible haematuria; VH: Visible haematuria

Table 3.4: Incidence of malignancy stratified according to age groups. NICE recommended age thresholds for haematuria investigations are shaded. 3.4A: Male. 3.4B: Female

3.4A

	Visible haematuria, n (%)					Non-visible haematuria, n (%)				
Age groups	**Total patients**	**All urinary tract cancers**	**Bladder cancer**	**Renal cancer**	**UTUC**	**Total patients**	**All urinary tract cancers**	**Bladder cancer**	**Renal cancer**	**UTUC**
10-19	2	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)
20-29	19	1 (5.3)	1 (5.3)	0 (0)	0 (0)	2	0 (0)	0 (0)	0 (0)	0 (0)
30-39	44	0 (0)	0 (0)	0 (0)	0 (0)	7	0 (0)	0 (0)	0 (0)	0 (0)
40-44	47	3 (6.4)	2 (4.3)	0 (0)	1 (2.1)	20	1 (5.0)	1 (5.0)	0 (0)	0 (0)
45-49	77	2 (2.6)	2 (2.6)	1 (1.3)	0 (0)	33	0 (0)	0 (0)	0 (0)	0 (0)
50-59	280	19 (6.8)	13 (4.6)	5 (1.8)	1 (0.4)	81	1 (1.2)	1 (1.2)	0 (0)	0 (0)
60-69	331	46 (13.9)	37 (11.2)	5 (1.5)	3 (0.9)	125	5 (4.0)	5 (4.0)	0 (0)	0 (0)
70-79	514	109 (21.2)	95 (18.5)	8 (1.6)	4 (0.8)	163	9 (5.5)	9 (5.5)	0 (0)	0 (0)
80-89	261	63 (24.1)	52 (25.2)	3 (1.1)	3 (1.1)	66	7 (10.6)	6 (9.1)	1 (1.5)	0 (0)
90-99	33	5 (15.2)	5 (15.2)	0 (0)	0 (0)	7	1 (14.3)	1 (14.3)	0 (0)	0 (0)
Total	1,608	248 (15.4)	207 (12.9)	22 (1.4)	12 (0.7)	504	24 (4.8)	23 (4.6)	1 (0.2)	0 (0)

3.4B

	Visible haematuria, n (%)					Non-visible haematuria, n (%)				
Age groups	**Total patients**	**All urinary tract cancers**	**Bladder cancer**	**Renal cancer**	**UTUC**	**Total patients**	**All urinary tract cancers**	**Bladder cancer**	**Renal cancer**	**UTUC**
10-19	1	0 (0)	0 (0)	0 (0)	0 (0)	0	0 (0)	0 (0)	0 (0)	0 (0)
20-29	20	0 (0)	0 (0)	0 (0)	0 (0)	8	0 (0)	0 (0)	0 (0)	0 (0)
30-39	31	0 (0)	0 (0)	0 (0)	0 (0)	26	0 (0)	0 (0)	0 (0)	0 (0)
40-44	35	3 (8.6)	3 (8.6)	0 (0)	0 (0)	25	0 (0)	0 (0)	0 (0)	0 (0)
45-49	55	1 (1.8)	0 (0)	1 (1.8)	0 (0)	44	1 (2.3)	1 (2.3)	0 (0)	0 (0)
50-59	163	7 (4.3)	1 (0.6)	3 (1.8)	3 (1.8)	155	2 (1.3)	2 (1.3)	0 (0)	0 (0)
60-69	174	16 (9.2)	13 (7.5)	1 (0.6)	2 (1.1)	206	4 (1.9)	3 (1.5)	0 (0)	0 (0)
70-79	153	23 (15.0)	18 (11.8)	4 (2.6)	1 (0.7)	190	4 (2.1)	2 (1.1)	2 (1.1)	0 (0)
80-89	58	10 (17.2)	9 (15.5)	1 (1.7)	0 (0)	81	4 (4.9)	2 (2.5)	2 (2.5)	0 (0)
90-99	14	5 (35.7)	4 (28.6)	0 (0)	1 (7.1)	5	0 (0)	0 (0)	0 (0)	0 (0)
Total	704	65 (9.2)	48 (6.8)	10 (1.4)	7 (1.0)	740	15 (2.0)	10 (1.4)	4 (0.5)	0 (0)

3.3.5 Bladder cancer histology according to haematuria presentation

Table 3.5 describes the histopathological breakdown of bladder cancers diagnosed by presentation of VH and NVH. Of the 288 bladder cancers confirmed following histopathological analysis, 88.5% of patients presented with VH. Grade 2 and 3 cancers accounted for 253 (87.8%) cancers and intermediate and high-risk bladder cancer was diagnosed in 109 (38.0%) and 145 (50.5%) patients respectively. MIBC was diagnosed in 135 (18.2%) patients with primary isolated CIS accounting for 34 (11.8%) of cases. UCC was diagnosed in 276 (92.3%) Squamous cell carcinoma and adenocarcinoma of the bladder were diagnosed in 6 (2.0%) patients. Other rare bladder cancers identified included giant cell carcinoma (n=1), amyloid (n=1) and non-Hodgkin's lymphoma of the bladder (n=1). All non-UCC bladder cancer presented with VH. Five cases had a diagnosis of benign papilloma.

Although bladder cancers diagnosed following a presentation of NVH accounted for only 33 (11.4%) of all bladder cancers, 18 (54.5%) of these patients had a diagnosis of grade 3 bladder cancer with 19 (57.6%) patients classified as high-risk disease. A total of 10 (30.3%) patients had a diagnosis of MIBC.

Table 3.5: Histopathological results following transurethral resection of bladder tumour stratified according to type of haematuria

	Any haematuria (n=288)	Visible haematuria (n=255)	Non-visible haematuria (n=33)
Grade, *n (%)*			
G1	34 (11.8)	27 (10.6)	7 (21.2)
G2	118 (41.1)	110 (43.3)	8 (24.2)
G3*	135 (47.1)	117 (46.1)	18 (54.5)
TMN stage, *n (%)*			
CIS*	3 (1.0)	3 (1.2)	0 (0)
pTa	174 (60.6)	158 (62.2)	16 (48.5)
pT1*	58 (20.2)	51 (20.1)	7 (21.2)
≥pT2*	52 (18.2)	42 (16.5)	10 (30.3)
papillary NMIBC + CIS	34 (11.8)	30 (11.8)	4 (12.1)
Number of tumours, *n (%)*			
1	220 (73.8)	196 (76.9)	24 (72.7)
≥2	46 (15.4)	41 (16.1)	7 (21.2)
Not known	32 (10.7)	18 (7.1)	2 (6.1)
Histology subtype, *n (%)*			
TCC	276 (92.3)	244 (91.7)	33 (100.0)
Adenocarcinoma	2 (0.7)	2 (0.8)	0 (0)
Squamous cell	4 (1.3)	4 (1.5)	0 (0)
Other**	3 (1.0)	3 (1.1)	0 (0)
Disease risk, *n (%)*			
Low	33 (11.5)	26 (10.2)	7 (21.2)
Intermediate	109 (38.0)	102 (40.2)	7 (21.2)
High	145 (50.5)	126 (49.6)	19 (57.6)

CIS = carcinoma in situ; NMIBC = non–muscle-invasive bladder cancer; TCC = transitional cell carcinoma; TNM = tumour, node, and metastasis.

* signifies high risk disease

**Other tumours comprise of a giant cell cancer, amyloid and non-Hodgkin's lymphoma

3.3.6 Comparison of different haematuria guidelines to identify urinary tract cancers

Table 1.1 summarises six haematuria guideline recommendations, all of which differ considerably. NICE recommends referring patients ≥45 years following a presentation of VH and ≥60 years following a presentation of NVH; indicated by shaded area in Tables 3.4A & 3.4B (2). In total, 600 patients (16.9%) were referred below the recommended age threshold for VH (n=199) or NVH (n=401). In this group, a diagnosis of urinary tract cancer was established in 11 (1.8%) patients, of which 10 patients had a diagnosis of bladder cancer and one with UTUC. The incidence of cancer in VH patients <45 years was 3.5% while patients <60 years with NVH had an incidence of 1.0%. Adopting the previous BAUS consensus statement which recommended investigating all patients with VH and patients ≥40 years with NVH would have identified all urinary tract cancers (12). Similarly, the AUA recommendations which stipulate investigating all VH patients and microscopic haematuria cases ≥35 years, would also detect all urinary tract cancers (23). Swedish NVH guidelines which recommend not investigating NVH will fail to detect 38 urinary tract cancers with an incidence rate of 3.1% (30).

Analysis of cancers detected below the NICE recommended age threshold for investigation of VH showed that one case was MIBC and a further four where high or intermediate risk NMIBC. Of the four bladder cancers presenting with NVH, one patient had G3 pT1 with three intermediate or high-risk NMIBC. Overall, 70% of bladder cancers which would have been missed were intermediate or high risk.

3.4 Discussion

This chapter underpins the importance of investigating patients presenting with haematuria to detect urinary tract cancer. To my knowledge, this is the first study to report cancer incidences following a presentation of either VH or NVH in the context of updated NICE guidance. I recruited cases across 40 hospitals to capture a contemporary perspective of UK haematuria referral pattern from primary care and the detection of urinary tract cancer in secondary care. The incidence of urinary tract cancer following a presentation of VH was 13.5% (male: 15.4% vs female: 9.2%) and NVH was 3.1% (male: 4.8% vs female: 2.0%). The finding that urinary tract cancers were detected in 1.8% of cases referred outside the NICE recommend age limit is of relevance, as we show that the incident rate of cancer in patients <45 years with VH was 3.5% and 1.0% in patients with NVH aged between 40-59 years.

This study provides valuable insight into the appropriateness of the recommendations to refer patients following a presentation of haematuria based on age thresholds. Despite guidance being issued over a year prior to commencement of this study, 600 patients (16.9%) were referred outside the NICE recommended age threshold which suggest that GPs may not be up to date with NICE recommendations and referral practice extended beyond NICE defined age thresholds. I highlight that 12.8% of all urinary tract cancers detected following a presentation of NVH and 2.2% of cancers detected following a presentation of VH would not have been detected if NICE guidance were adhered to. It is possible that in time, a greater awareness of the criteria for the two-week wait cancer referral pathway and scrutiny by commissioning groups based on the current NICE guidance will restrict the referral of cases outside guidelines. Data

presented here would suggest that this would lead to a delay in detection of cancer either through non-urgent referrals, late presentation following referral only after recurrent episodes of bleeding or the emergence of VH following an initial presentation of NVH.

I report that 70% of bladder cancers detected below the age threshold were either intermediate or high risk NMIBC representing significant disease. Early diagnosis of high risk and muscle invasive bladder cancer is important as a delay in diagnosis has been shown to impact patient survival (145-147). Approximately 18% of patients diagnosed with bladder cancer consult their general practitioner ≥3 times prior to referral for investigation suggesting that the need for inclusive recommendations is necessary to enable prompt referral for investigations (148). As highlighted, there remains a lack of global consensus on the requirement to investigate VH and NVH.

Establishing a minimum PPV of a symptom or clinical sign associated with the presence of cancer is important to determine which patients would benefit from investigations. NICE suggests that a clinical sign or symptom associated with a ≥3% risk for cancer should prompt referral for diagnostic tests. In contrast, the AUA seeks to define a threshold resulting in the detection of 99% of cancer (2, 18). Clearly, these results suggest a case for the investigation of all patients with VH where 7 of the 199 patients <45 years investigated had a diagnosis of cancer. This corresponded to a number needed to screen of 28 patients to detect one case acknowledging this represents incidence rates in secondary care. The incidence of urinary tract cancer is much lower in patients presenting with NVH, If the age threshold of screening for patients with NVH was lowered from 60 years to 40 years for patients, assuming 224 patients were screened to identify 5 urinary

tract cancers, the number needed to screen was 44.8 cases to identify a case of urinary tract cancer. Further, out results suggest that significant number of patients with NVH may still harbour significant disease.

The importance of patient preference has recently been highlighted using a vignette study to explore the likelihood that patients would want diagnostic tests if there was a risk of cancer diagnosis (149). Banks and colleagues showed that 85% of patients would want referral for investigation for a symptom attributing a 1% risk of cancer, even if invasive testing is required such as colonoscopy for colon cancer (149).

An important question which cannot be answered by this study is what the acceptable age threshold should be to recommend the investigation of NVH. I report that the overall incidence of urinary tract cancers in female patients presenting with NVH is 2.0% (bladder cancer incidence of 1.4%) but these patients would be investigated despite the fact that cancer risk is lower than the 3% recommended by NICE to prompt investigations. Hence, based on the same argument, investigating patients aged 40-59 years presenting with NVH should be investigated based on an incidence of 1.4% of urinary tract cancer in this patient cohort. This is based by knowledge that a significant number of cancers diagnosed following a presentation of NVH are clinically significant. The previous BAUS Consensus Statement for haematuria assessment recommended investigation of asymptomatic NVH in patients aged ≥40 years (12). Reverting to this threshold will allow the detection of all cancers presenting with NVH in our cohort. The AUA recommended threshold of ≥35 years for NVH would similarly identify all cancers but clearly increase the number of patients investigated (18).

3.5 Limitations

An important limitation of the study is that cases where accrued by sampling individual haematuria clinics, rather than recruiting all consecutive patients during a defined time period. However, to mitigate a potential selection bias based on diagnosis, all patients were recruited prior to cystoscopy to exclude any selection bias. While we acknowledge that some patients may be recruited following an upper tract scan due to the one-stop haematuria clinic pathway, results of the scans were not known to research nurses who were involved in patient recruitment. A further consideration is that the incidence of urinary tract cancer in patients with haematuria reported in this study reflects the detection rate in secondary care, which will be inevitably higher than the actual incidence in primary care due to case selection for referral. In formulating recent policy, the NICE recommendations were drawn using data from primary care which itself introduces a bias. The low incidence cancer rates of bladder cancer reported using primary care medical records will include patients with haematuria associated with UTI and opportunistic one off dip stick testing neither of which would normally trigger referral for investigations (150). There remain no prospective observational studies of patients recruited at the time of presentation or detection of haematuria in the primary care setting.

It must also be considered that the current study did not assess for the presence of dysuria or raised WCC at the time of referral and, both are required to be present at the time of referral based on NICE guidance. The ability to determine if either measure was present or absent could have altered the outcome and potentially result in a greater number of missed urinary tract cancers.

3.6 Conclusions

This study suggests that patients with VH should be investigated regardless of age. A decision to investigate NVH should reflect public health policy and patients' choice. Nevertheless, adopting the NICE recommendations will result in missed cancers and there remains a lack of consensus across guideline bodies. It is likely that an international consensus would aid physician decision making and the selection of appropriate patients for the investigation of haematuria.

CHAPTER 4 : DIAGNOSTIC ABILITY OF IMAGING, CYSTOSCOPY AND URINARY CYTOLOGY TO IDENTIFY URINARY TRACT CANCER

4.1 Introduction

As reported in Chapter 3, the risk of urinary tract cancer in patients presenting with VH is 13.5%, and by comparison the risk of malignancy is 3.1% for patients presenting with NVH (151). Older patients and those with a smoking history are significantly more likely to have a cancer diagnosis. Bladder cancer is the most common cancer accounting for 81.8% of cancers diagnosed. The overall incidence of upper tract cancers albeit low at 1.5% necessitates imaging especially in patients presenting with VH.

The combination of cystoscopy and upper tract imaging is essential for investigating patients with haematuria. While there is a resounding consensus that cystoscopy remains the investigation of choice to visualise the bladder, there is a lack of consensus for the optimal upper tract imaging modality. RBUS and CTU are the most commonly used imaging modalities. The use of intravenous urography has largely been superseded by CTU. The AUA recommends using CTU for both VH and NVH while the NICE and the American College of Physicians guidelines do not specify a recommended imaging modality (2, 18, 152).

CTU has the highest diagnostic performance to identifying upper tract disease. Meta-analysis suggest CTU achieves a sensitivity of 96% (88-100%) and specificity of 99% (98-99%) for the detection of UTUC (153). However, the diagnostic performance of CTU should be balanced against the risk attributed by intravenous contrast. Intravenous contrast administration is associated with a 3% risk of contrast induced nephropathy in high risk patients (eGFR: 30-59 ml/min/1.73m^2) and prophylaxis by hydration has been shown to be ineffective in reducing this risk (154, 155). In addition, exposure to ionising radiation itself is

carcinogenic and although rare, there is a risk of anaphylactic reaction attributed to intravenous contrast (156, 157).

Urine cytology is a frequently used test which is available in most hospitals (158). It has a high specificity but highly variable sensitivity (38-84%) for high grade disease and low sensitivity for low grade bladder cancer (20-53%) (159). Hence, even with a high negative predictive value (NPV) of 92%, urine cytology cannot be recommended as a standalone test (160).

There is no consensus among guideline bodies regarding the inclusion of urine cytology for assessment of haematuria. The NICE bladder cancer guidelines do not specify investigations for patients presenting with haematuria but recommend that patients with a new diagnosis of bladder cancer should have urine cytology or an alternative urinary biomarker (such as UroVysion using fluorescence in-situ hybridization [FISH], ImmunoCyt or NMP22) in addition to cystoscopy (161). The AUA guidelines suggest that cytology may be useful for patients with persistent NVH following a negative workout or in patients with a high risk of CIS (irritative voiding, current/ past tobacco use, chemical exposure) (18). Such confusing and inconsistent recommendations results in significant variation in clinical practice across centres and countries.

In this chapter, I report the diagnostic ability of CTU, RBUS, cystoscopy and urine cytology to identify urinary tract cancer. I aim to determine the ideal combination of tests required for the investigation of haematuria. Results from this chapter have been recently published in the journals *Journal of Urology* and *British Journal of Urology International* (162, 163).

4.2 Methods

4.2.1 Patient selection

Cases included in this study comprise of the same patient cohort described in Chapter 3.2.1. For the assessment of urine cytology, 9 hospitals were identified in which urine cytology was performed as part of standard of care tests in addition to cystoscopy and upper tract imaging for patients evaluated for haematuria.

4.3.2 Interventions

Clinical evaluation of patients has been previously described in Chapter 3.2.2. The decision to perform RBUS, CT urogram or both form of imaging modality of dependent on local guidelines or clinician discretion. In addition to interventions performed as described previously, suboptimal imaging for the visualisation of the bladder was defined as scans where the bladder was reported as under filled or inadequate assessment of the bladder due to artefact from metal prosthesis or implants.

Urine samples for cytopathological assessment were sent to the receiving hospital laboratory of respective hospitals where they were centrifuged, and a monolayer of cells were prepared on a glass slide. Cells were then stained with Papanicolaou staining and examined by microscopy by a cytopathologist. Urinary cytology was classified to 1) suspicious/ consistent with neoplastic cells, 2) atypical cells or 3) negative for cancer. A positive urine cytology was defined as a score of ≥3 on the Paris System for reporting of urinary cytology (164). Urine samples with inadequate cellular content were excluded from analysis. Analysis

reporting the combined diagnostic performance of urine cytology and imaging is determined based on the ability of either urine cytology of imaging to detect bladder cancer or UTUC.

4.2.3 Statistical analysis

Continuous data such as mean, median, interquartile range and 95% confidence interval were reported using descriptive statistics. Categorical variables were compared using Chi-square test. T-test was used to compare continuous variables. Normal distribution was assumed. Sensitivity, specificity, PPV and NPV were calculated for correct identification of bladder cancer or upper tract cancers. SPSS v22 (IBM Corp, Armonk, New York, USA) was used to perform all statistical analysis. Statistical significance was set at p value <0.05. This study was registered with ClinicalTrials.gov, number NCT02676180.

4.3 Results

4.3.1 Imaging for the detection of urinary tract disease

4.3.1.1 Patient demographics

A Flow diagram of patients recruited into the study is shown in Figure 4.1. Patient demographics are shown in Table 4.1. The overall incidence of urinary tract cancer was 10.0% (bladder cancer 8%, renal cancer 1%, UTUC 0.5%). The full break down of urinary tract disease has been previously reported in Chapter 3. RBUS was performed on 2,166 (60.9%) patients and CTU on 1,693 (47.6%) patients, 470(13.2%) patients had both RBUS and CTU.

Figure 4.1: CONSORT diagram of patients with breakdown of upper tract imaging

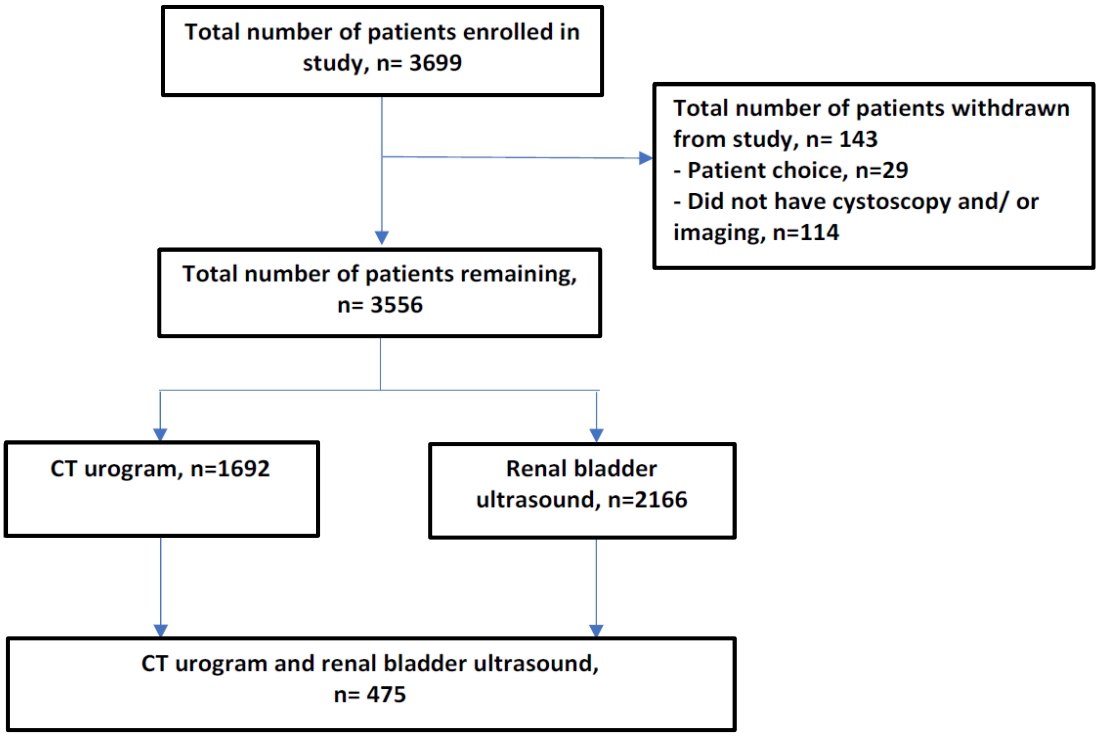


Table 4.1: Patient demographics according to type of haematuria

	All patients (n=3,556)	VH (n=2,311)	NVH (n=1,245)	p value
Age (median, IQR)	67.7 (57, 76)	68.1 (56.4, 76.2)	67.0 (56.9, 75.0)	0.568
Gender, n (%):				<0.001
Male	2,112 (59.4)	1,607 (69.5)	505 (40.6)	
Female	1,444 (40.6)	704 (30.5)	740 (59.4)	
Ethnicity, n (%):				0.235
White	3,080 (86.6)	2,013 (87.1)	1,067 (85.7)	
South Asian	86 (2.4)	57 (2.5)	29 (2.3)	
Afro-Caribbean	51 (1.4)	36 (1.6)	15 (1.2)	
Oriental	15 (0.4)	8 (0.3)	7 (0.6)	
Mix	31 (0.9)	20 (0.9)	11 (0.9)	
Other	23 (0.6)	18 (0.8)	5 (0.4)	
Not known	271 (7.6)	159 (6.9)	111 (8.9)	
Smoking history, n (%):				0.739
Non-smoker	1,528 (43.0)	991 (42.9)	537 (43.1)	
Current/ ex-smoker	1,896 (53.3)	1,240 (53.7)	656 (52.7)	
Not known	132 (3.7)	80 (3.4)	52 (4.2)	
Any urinary tract cancer, n (%)	354 (10.0)	315 (13.6)	39 (3.1)	<0.001
Bladder cancer, n (%)	288 (8.1)	255 (11.0)	33 (2.7)	<0.001
Renal cancer, n (%)	37 (1.0)	32 (1.4)	5 (0.4)	0.006
Upper tract UCC, n (%)	18 (0.5)	18 (0.8)	(0)	0.002
Renal calculi, n (%)	270 (7.6)	215 (9.3)	55 (4.4)	<0.001

IQR: interquartile range, NVH: non-visible haematuria, UCC: urothelial cell carcinoma, VH: visible haematuria

4.3.1.2 Diagnostic performance of RBUS and CTU for the detection of upper tract disease

Of the 2,166 patient who had RBUS, the incidence of RCC and UTUC were 0.6% (n=14) and 0.3% (n=7) respectively. CTU was performed on 1,692 patients and the detected incidence of RCC and UTUC was 2.1 (n=35) and 1.1% (n=18) respectively. Table 5.2 shows the diagnostic ability of RBUS and CTU at detecting upper tract disease.

RBUS identified 12 of 14 (85.7%) renal cancers and misclassified one renal cancer as a UTUC increasing the sensitivity of detecting cancer to 92.9% with a NPV of 99.9%. The sensitivity of RBUS for the detection of UTUC was poor (14.3%). Three patients were misclassified as renal cancer and one UTUC diagnosed on RBUS was renal cancer on histology suggesting a sensitivity of 62.5% to detect cancer with a NPV of 99.9%.

Given that a suspicious CTU for renal cancer or UTUC would result in further test to evaluate the lesion and that patients with a negative CTU would be discharged, the sensitivity and NPV for CTU cannot be determined. The PPV of CTU to diagnose renal cancer was 94.6%, two suspicious lesions on imaging were benign on histology. CTU had a PPV of 72.0% for the diagnosis of UTUC, with 19 suspected UTUC cases correctly identified. Three suspected UTUC were histologically confirmed renal cancers, suggesting a PPV of cancer of 88.0%. Ureteroscopy with / without biopsy did not confirm cancer in 3 cases with suspicious imaging. Diagnostic performance of RBUS at identifying renal calculi was poor using CT as a reference standard with a sensitivity, specificity, PPV and NPV of 34.0%, 97.9%, 65.4% and 92.7% respectively.

4.3.1.3 Diagnostic ability of RBUS, CTU and cystoscopy at identifying bladder cancer

Table 4.2 reports the diagnostic ability of RBUS, CTU and cystoscopy at detecting bladder cancer. The diagnostic accuracy for RBUS to identify bladder cancer was sensitivity: 50.7%, specificity 99.3%, PPV 84.3% and NPV 96.5%. CTU was better than RBUS at identifying bladder cancer. The sensitivity, specificity, PPV and NPV of CTU to identify bladder cancer was 80.8%, 97.0%, 78.9% and 97.3%. Excluding suboptimal scans, the diagnostic ability of RBUS and CTU to detect bladder cancer improved.

The sensitivity and NPV of cystoscopy cannot be determined as patients with a normal flexible cystoscopy were discharged without follow-up cystoscopy. Using histopathological confirmation of tumour as reference, the specificity of flexible cystoscopy was high at 98.3% with a PPV of 84.0%.

Table 4.2: Comparison of RBUS, CTU and flexible cystoscopy to diagnose bladder cancer, renal cancer and UTUC

Diagnostic test	Reference standard	Diagnostic accuracy				
		Sensitivity, % (95% CI)	**specificity, % (95% CI)**	**PPV, % (95% CI)**	**NPV, % (95% CI)**	**Area under the curve**
RBUS (n=2166)	Histopathological confirmation of UTUC	14.3 (0.9-49.4)	100 (99.8-100.0)	50.0 (3.8-96.2)	99.7 (99.4-99.9)	0.571
CTU (n=1692)	Histopathological confirmation of UTUC		99.6 (99.2-99.8)	72.0 (52.8-86.9)		
RBUS (n=2166)	Histopathological confirmation of renal cancer	85.7 (62.1-97.5)	99.2 (98.8-99.5)	41.4 (24.8-59.5)	99.9 (99.7-100.0)	0.925
CTU (n=1692)	Histopathological confirmation of renal cancer		99.9 (99.6-100.0)	94.6 (84.2-99.1)		
RBUS (n=475)	CTU to diagnose renal calculi	34 (21.9-47.7)	97.9 (96.2-99.0)	65.4 (46.3-81.6)	92.7 (90.0-94.8)	0.659
RBUS (n=2166)	Histopathological confirmation of bladder cancer	50.7 (42.7-58.7)	99.3 (98.9-99.6)	84.3 (75.8-90.8)	96.5 (95.6-97.2)	0.750
Unoptimised RBUS excluded (2090)		63.6 (54.7-71.9)	99.3 (98.9-99.6)	84.3 (75.8-90.8)	97.9 (97.2-98.4)	0.814
CTU (1692)	Histopathological confirmation of bladder cancer	80.5 (74.8-85.4)	97.0 (96.1-97.8)	79.3 (73.6-84.4)	97.2 (96.3-98.0)	0.887
Unoptimised CTU excluded (1615)		83.6 (78.1-88.3)	97.0 (96.1-97.8)	80.0 (74.2-85.0)	97.7 (96.8-98.4)	0.903
Cystoscopy (n=3556)	Histopathological confirmation of bladder cancer		98.3 (97.9-98.7)	84.0 (79.7-87.5)		

CTU: CT Urogram; PPV: positive predictive value; NPV: negative predictive value; RBUS: renal bladder ultrasound

4.3.2 Diagnostic ability of urinary cytology of for the detection of transitional cell carcinoma

4.3.2.1 Patient demographics

Of the 3,556 patients recruited, urine cytology was performed on 567 patients (15.9%) as a routine test in 9 of the 40 participating hospitals, of which 8 were district general hospitals. In all cases, urine cytology was submitted in addition to cystoscopy and upper tract imaging. Patient demographics of the 567 patients are shown in Table 4.3. Median age was 67.7 years and 395 (69.7%) and 172 (30.3%) patients were investigated following a presentation of VH or NVH respectively. In total, 39 (6.9%) bladder cancers and 8 (1.4%) UTUC were identified in this cohort. Median time interval between a positive urine cystoscopy to endoscopic tumour resection was 27 (IQR: 21.3-33.8) days.

4.3.2.2 Diagnostic performance of urine cytology

Thirteen urinary samples (2.3%) were excluded due to inadequate urinary cellular content for cytology analysis (Figure 4.2). The overall accuracy of a positive / atypical urine cytology for the diagnosis of bladder cancer or UTUC was: sensitivity 43.5%, specificity 95.7%, PPV 47.6% and NPV 94.9% (Table 4.4) with an AUC of 0.713. The diagnostic ability of a positive / atypical urine cytology to identify high risk disease was marginally better: sensitivity 57.7%, specificity 94.9%, PPV 35.7% and NPV 97.9% with an AUC of 0.688 (Table 5.4). Selecting patients with VH only had a similar diagnostic performance (Table 5.4). Sub analysis to examine the role of atypical urine cytology showed a low sensitivity of 6.0% while a positive urine cytology achieved a specificity of 98.4% with a ROC of 0.856 (Table 5.4). In total, 26 (52.3%) patients had a false negative result for

urine cytology, of which 21 were bladder cancers and 5 were UTUC. Bladder cancers missed according to grade and stage were: 4 (19%) ≥ pT2, 2 (9.5%) G3 pT1, 10 (47.6%) G3/2 pTa and 5 (23.8%) G1 pTa. High risk cancer accounted for 38% of patients. No bladder cancer or UTUC were diagnosed based on a suspicious urinary cytology test alone. Stratifying patients according to smoking history did not change the performance of urine cytology.

Figure 4.2: CONSORT diagram of patients where urinary cytology was performed

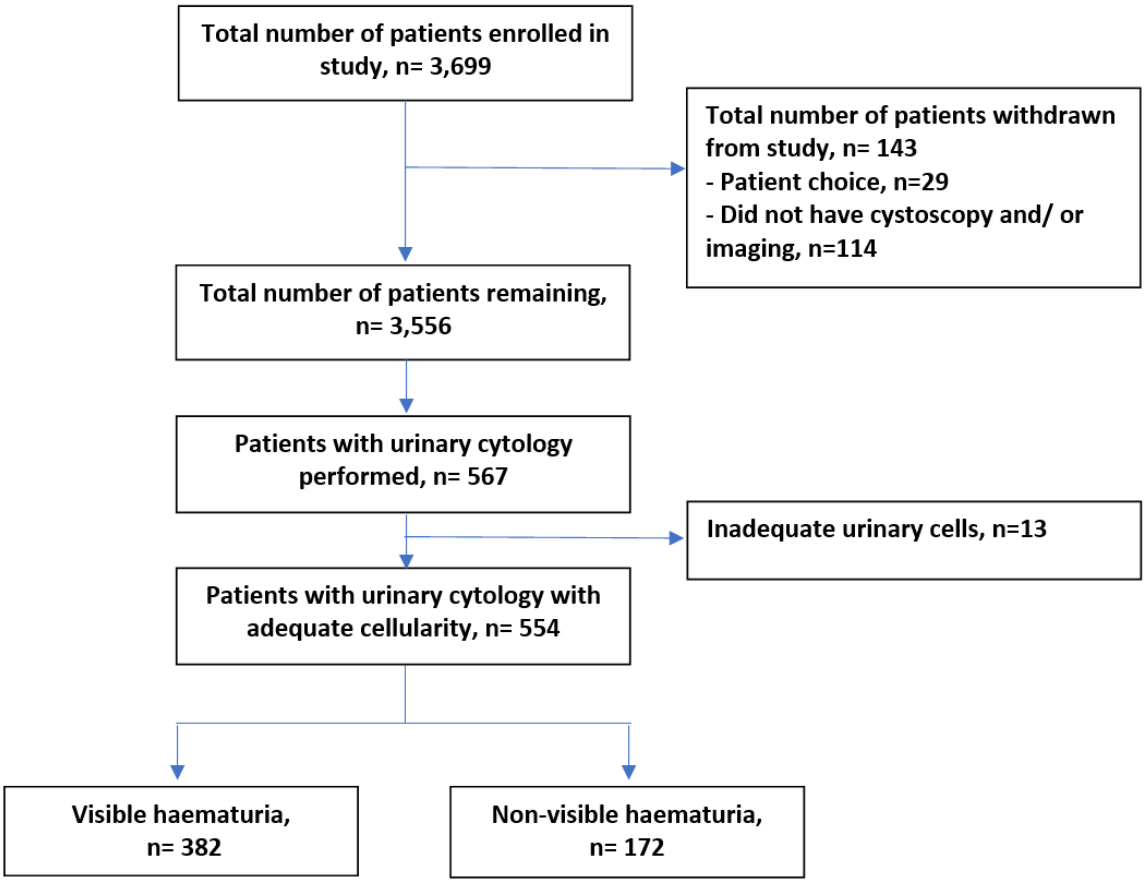


Table 4.3: Patient, cytology and histopathological characteristics

Variables	n=567 (%)
Age, median (IQR) years	67.7 (55.6, 75.7)
Gender, n (%):	
Male	342 (60.3)
Female	225 (39.7)
Smoking history, n (%):	
Non-smoker	240 (42.3)
Current smoker	87 (15.4)
Previous smoker	231 (40.7)
Not known	9 (1.6)
Type of haematuria, n (%):	
Visible	395 (69.7)
Non-visible	172 (30.3)
Urine cytology, n (%):	
Inadequate cellular content/ non-diagnostic	13 (2.3)
Negative	512 (90.3)
Atypical	21 (3.7)
Suspicious/ consistent with neoplastic cells	21 (3.7)
Bladder cancer, n (%)	39 (6.9)
Upper tract UCC, n (%)	8 (1.4)
Bladder cancer grade, n (%):	
G1	6 (15.4)
G2	14 (35.9)
G3	19 (48.7)
Concurrent CIS, n (%)	6 (15.4)
Bladder cancer stage, n (%):	
CIS	0 (0)
pTa	24 (61.5)
pT1	8 (20.5)
≥pT2	7 (17.9)

5.3.2.3 Outcome of patients with suspicious urine cytology with normal cystoscopy and upper tract imaging

Twenty two patients had a positive urine cytology despite a normal cystoscopy and upper tract imaging. Twelve (54.5%) patients had a further diagnostic procedure in for a form of ureteroscopy with / without biopsy (n=5) or an interval cystoscopy (n=7). No bladder cancer, ureteric or renal pelvis UTUC were identified. Five (22.7%) patients had a repeat urine cytology which was normal. Urine cytology in two (9.1%) patients were reported as scanty mild atypia cells

and a further three (13.6%) patients were lost to follow-up. No patient had a subsequent diagnosis of cancer following further investigations. At the point of this analysis, all patients had a minimum of one year follow-up.

4.3.2.4 Diagnostic performance of urinary cytology in combination with upper tract imaging

The combination of urine cytology with urinary tract imaging significantly increases the diagnostic performance to detect bladder cancer (Table 4.4). The combination of urine cytology with CTU (sensitivity: 92.3%, specificity: 94.9%) was superior when compared to urine cytology with RBUS (sensitivity: 66.7%, specificity: 96.7%). In comparison, CTU alone achieved a diagnostic performance of sensitivity 80.5%, specificity 97.0%, PPV 79.3% and NPV 97.2% while RBUS had a sensitivity of 50.7%, specificity of 99.3%, PPV 84.3% and NPV of 96.5%.

Table 4.4: The diagnostic accuracy of urinary cytology to diagnose bladder cancer with/ without upper tract transitional cell carcinoma stratified according to different patient cohorts.

Test	Patient cohort	Diagnostic accuracy				
		sensitivity	**specificity**	**PPV**	**NPV**	**ROC**
Positive/ atypical urine cytology	All patients	43.5 (29.8-57.9)	95.7 (93.7-97.2)	47.6 (33.0-62.5)	94.9 (92.8-96.6)	0.713 (0.615-0.811)
	Visible haematuria	44.2 (30.0-59.0)	94.7 (92.0- 96.7)	51.4 (35.6-67.0)	93.0 (90.0- 95.4)	0.722 (0.619-0.825)
	High risk bladder cancer	57.7 (38.7-75.3)	94.9 (92.8-96.6)	35.7 (22.4-50.7)	97.9 (96.3-98.9)	0.688 (0.567-0.769)
Positive urine cytology	All patients	38.2 (23.2-55.0)	98.4 (97.0-99.3)	61.9 (40.7-80.4)	95.9 (93.9-97.4)	0.856 (0.747-0.964)
Atypical urine cytology	All patients	6.0 (5.3-33.4)	96.7 (94.8-98.0)	19.0 (6.3-38.9)	95.9 (93.9-97.4)	0.570 (0.433-0.707
Positive/ atypical urine cytology or suspicious CTU suggestive of bladder cancer or UTUC	All patients	90.2 (78.8-96.9)	94.9 (91.9-97.0)	71.2 (58.0-82.2)	98.6 (96.7-99.6)	0.849 (0.773-0.924)
	Visible haematuria	92.3 (81.2-98.0)	94.4 (91.1-96.8)	72.0 (58.7-83.1)	98.7 (96.8-99.7)	0.854 (0.778-0.930)
Positive/ atypical urine cytology or suspicious RBUS suggestive of bladder cancer or UTUC	All patients	66.7 (34.5-90.5)	96.7 (94.0-98.5)	42.9 (19.8-68.3)	98.8 (96.8-99.7)	0.708 (0.535-0.882)
	Visible haematuria	66.7 (34.5-90.5)	96.6 (92.3- 98.9)	60.0 (30.0-85.4)	97.4 (93.5-99.4)	0.787 (0.597-0.977)

CTU: CT urogram, NPV: negative predictive value, PPV: positive predictive value, RBUS: renal, bladder ultrasound, ROC: receiver operator characteristics, UTUC: Upper tract urothelial cancer

4.4 Discussion

In this chapter, I report that RBUS has a diagnostic sensitivity of 92.9% for the identification of renal cancer. However, it had only a 62.5% sensitivity for the identification of UTUC (including 3 cancers diagnosed as renal cancer and one UTUC which was renal cancer on histology) and missed 3 of 8 UTUC (37.5%). As reported in Chapter 3, the incidence of UTUC is low at 0.5% in patients with haematuria. The fact that no UTUC was identified following a presentation of NVH suggest that RBUS should be sufficient to assess the upper urinary tract in patients presenting with NVH. With regards to the role of urine cytology, the diagnostic ability of urine cytology was poor even for high grade bladder cancer and regardless of risk group stratification such as those with VH. In addition, there were 22 (4.3%) false positive patients who had urine cytology, 54.5% of patients were investigated further with invasive diagnostic tests and no additional cancer was diagnosed.

I report that cystoscopy has a specificity of 98.3% with a PPV of 83.9% suggesting that conventional imaging modalities cannot replace cystoscopy. Even after excluding suboptimum scans, the accuracy of RBUS to detect bladder cancer was poor, with a sensitivity of 63.6% and specificity of 99.3%. CTU had a higher diagnostic accuracy to identify bladder cancer (sensitivity: 83.6%, specificity: 97.0%) but not sufficient to replace cystoscopy.

4.4.1 Imaging for the detection of urinary tract cancer

It is estimated that the incidence of NVH is as high as 2.5% of the population and rises to as high as 18% in male patients ≥70 years (6, 165). However, the majority of these cases do not have a sinister identifiable cause for NVH. CTU has been shown to be superior at identifying UTUC compared to RBUS (3, 153). RBUS may miss small ureteric tumours, which are too small to cause luminal occlusion. This can result in a false negative, as in cases where no hydronephrosis is identified, no further imaging may be performed. The operator dependent nature of RBUS may also result in missed small renal pelvis UTUCs. While CTU is superior at identifying UTUC, the risk of UTUC in patients presenting with NVH is low suggesting that there is no benefit for CTU over RBUS (153).

RBUS has been shown to detect renal cancer with a high sensitivity although a small number of cases are false positive (n=14). These false positive cases would have a second scan, typically a renal protocol CT which will better characterise a renal mass. Hence, the approach of performing cystoscopy with RBUS instead of CTU to investigate the upper tracts of patients presenting with NVH should be the recommended upper tract imaging of choice. I acknowledge that RBUS has a poor sensitivity for the identification of renal calculi. Hence, I propose that patients presenting with symptoms suggestive of renal colic such as flank pain would benefit from RBUS with non-contrast CTKUB or CTU. I acknowledge that replacing CTU with RBUS for patients with NVH would potentially miss asymptomatic renal calculi with no hydronephrosis. However, I believe such patients would be uncommon and identifying such a patient will be at the expense of subjecting a high number of patients to CTU which would yield negative results.

In an ideal world, all patients should be investigated with the best diagnostic test available. However, risk of adverse events, low incidence of disease in the specific patient cohort as well as the high cost of diagnostic test would make this economically questionable. In the case of NVH, the disease specific incidence of UTUC is low (0%) and below the 3% threshold for diagnostic investigation used by NICE and the 1% suggested by the AUA (2, 18). Further, iodinated contrast carries a low but significant risk of allergic reaction which can be life threatening (157). Ionising radiation from CTU is 4 mSv with is 200 times that of a standard chest X-ray (166). The cumulative exposure to ionising radiation has been shown to account for 0.6-0.9% of cancer diagnosed (156). In fact, a recent report suggests that subjecting patients with NVH to CTU may cause more secondary malignancies attributed by CT-associated radiation than the number of upper tract cancers missed by RBUS (167).

Recently, a cost-effectiveness analysis has recommended using RBUS instead of CTU for the evaluation of NVH patients (168). A comparison of four diagnostic approaches comprising of CTU alone, cystoscopy alone, CTU with cystoscopy and RBUS with cystoscopy suggest that the RBUS with cystoscopy represents the most cost-effective combination at $53,810 per cancer detected. Replacing RBUS with CTU for the investigation of NVH will cost $6,480,484 per cancer identified. It is estimated that using RBUS instead of CTU will result in cost savings of $390 million which is much needed in an era of escalating healthcare cost (169).

The role of cystoscopy to visualise the bladder remains the gold standard. Even after excluding suboptimal scans, a patient with a normal CTU or RBUS will still require cystoscopy due to a high risk of a false negative. The diagnostic

performance of imaging for the detection of bladder cancer is similar to the diagnostic ability of FDA approved urinary biomarkers for the detection of bladder cancer with a reported sensitivity of 57-82% and specificity of 74-88% (170). While larger tumours would be easily identifiable by imaging modalities, smaller tumours may be missed. It is likely that an optimised CTU, where the urinary bladder is well distended, and contrast has fully opacified the bladder lumen, will improve the diagnostic performance. However, such scans may be difficult to achieve in clinical practice.

While the majority of bladder lesions are considered cancer until proven otherwise, we report that a visual diagnosis of malignancy seems reliable and has a specificity of 98.3% following white light cystoscopy. In the setting of surveillance cystoscopy, low grade bladder cancer was identifiable from high grade cancers by urologists 99% of the time (171). Cystoscopy is operator dependent and the specificity for a more experienced cystoscopist will be higher. Hence, it is essential that suspicious bladder lesions be biopsied due to a high likelihood of malignancy. Bladder biopsy can be performed at the point of initial diagnosis with flexible cystoscopy and this can reduce the need for a general anaesthesia.

4.4.2 Diagnostic ability of urinary cytology of for the detection of transitional cell carcinoma

There have been two historic single institution reports on the role of urine cytology in the haematuria setting. Hofland and colleagues reported that urine cytology successfully identified cancer in 0.2% (n=2) which were missed on cystoscopy or imaging (172). The study by Mishriki and colleagues showed that 0.07% (n=2) of patients had a cancer detected solely by urine cytology (173). In the current study, urine cytology did not detect any additional cancers not already identified by imaging or cystoscopy and the results suggest that routine urine cytology has no added benefit for the assessment of haematuria.

Table 4.5 summarises the recommendation of the AUA, NICE, BAUS (subsequently replaced by NICE), NCCN, Canadian, Dutch and Japanese Urology Associations. With the exception of the previous BAUS haematuria recommendation, all other guidelines recommend the use of urinary cytology in selected patients presenting with haematuria. However, there is no consistency and the recommended patient groups that may benefit from urinary cytology varies between the guidelines (61).

Table 4.5: Comparison of recommendations on the use of urinary cytology.

AUA (18)	Cytology not recommended for asymptomatic NVH. In patients with persistent NVH following a negative work up or those with carcinoma *in situ* risk factors (irritative voiding, current/ past tobacco use, chemical exposure) cytology may be useful. No comment for VH
CUA (174)	All haematuria patients should have cytology. Those with negative investigations should have urinary cytology in conjunction with urinalysis and blood pressure checks at 6, 12, 24 and 36 months. No comment for VH
BAUS (12)	Cytology not part of VH or NVH investigations
NICE (161)	Role of cytology not commented for initial investigations. Cytology/ urinary biomarker or photodynamic diagnosis/ narrow band imaging in patients with suspected bladder cancer
NCCN (175)	Role of cytology not commented for initial investigations. Consider cytology for suspected bladder cancer
JUA (31)	Cytology recommended for VH. NVH without risk factors should have renal bladder ultrasound or cytology
DAU (176)	Cytology recommended in VH patients of any age or NVH >50 years following a negative work up

AUA: American Urology Association, BAUS: British Association of Urological Surgeons, CUA: Canadian Urology Association, DAU: Dutch Association of Urology, JUA: Japan Urology Association, NICE: National Institute for Health and Care Excellence, NCCN: National Comprehensive Cancer Network, NVH: non-visible haematuria

Although, urine cytology has a high specificity, reported sensitivity can range from 12-85% (159, 177). The proportion of high grade tumours, interobserver variability, sample preparation and differences in urine collection methods can explain this wide variation (178). Ideally, urine samples collected for cytology should include three daily mid-morning or random samples and be transferred to receiving laboratory in a timely manner (179). Where long delays are expected, an equal volume of 50% alcohol should be added to allow prompt fixation. Multiple urine voided samples have been shown to increase the sensitivity from 44% to 67% in a retrospective single institutional study (180). However, in clinical

practice, this is rarely performed and patients are often seen in busy one-stop haematuria clinic and only one voided urine sample is collected and used for both urinalysis and urine cytology.

Over time, different reporting criteria have been used when reporting urine cytology (181). However, none of these criteria has gained wide spread acceptance resulting in significant variation in reporting. Even when the same reporting criteria is used, significant intra-observer variability between cytopathologists remains (178, 181). Central review of 652 urine cytology specimens report a Kappa coefficient of between 0.36-0.45 for non-tertiary institutions (178).

In addition, a report of 'atypical' urine cytology represents a diagnostic conundrum. There is no consensus on the exact classification of atypical urine cytology. Published reports suggest that up to 23.2% of urine cytology are categorised as atypical (182). The prognostic value of atypical urine cytology is debateable. A retrospective analysis of 1320 patients with atypical urine cytology suggest that 21% of cases will develop malignancy with a mean follow-up of 155 days although others have questioned the significance of the atypical category (182, 183).

The cost of urine cytology is estimated to be £114.55 (2012 adjusted cost) based on a Health Technology Assessment (HTA) estimate (184). Flexible cystoscopy and ultrasound imaging are estimated to cost £401.88 and £83.85 respectively, which suggests that urinary cytology costs nearly 20% of the cost of haematuria investigation. No guidelines body recommends that urine cytology or any other urinary biomarkers can replace cystoscopy and direct visualisation of the bladder is recommended in patients with haematuria. Other commercially available

urinary biomarkers such as FISH, NMP22, ImmunoCyst and Cxbladder achieve a sensitivity of 57%-82% and specificity of 74%-88% which will miss a substantial number of bladder cancers with a high risk of a false positive result (185). The requirement for cystoscopy and upper tract imaging makes the need for cytology redundant. Given that white light cystoscopy has a sensitivity of >98% to diagnose bladder cancer, a positive urine cytology for malignancy is more likely to reflect a false positive than a missed tumour on cystoscopy (133).

CTU under ideal conditions has been shown to achieve a sensitivity of 95% and NPV of 98% suggesting that CTU can be used as a form of triage to refer patients directly for rigid cystoscopy where TURBT can be performed, bypassing the need for flexible cystoscopy (133). While the current study did not report as high a sensitivity and NPV for either CTU and RBUS for the detection of cancer, we report that the combination of urinary cytology with imaging results in an improved sensitivity for the detection of cancer, however, this improvement is not sufficient to replace cystoscopy (162).

While urine cytology improves the detection rate of cancer when combined with imaging, this increase in diagnostic performance is at the expense of the risk of false positives. We report that 22 (4.3%) of patients in this cohort had a positive cytology result despite a normal cystoscopy and upper tract imaging. None of these patients had a subsequent diagnosis of cancer. Twelve (54.5%) patients had further invasive tests such as ureteroscopy with / without ureteric urine sampling or an interval cystoscopy while others had a repeat urinary cytology which confirms the absence of cancer. All of these tests were triggered by a false positive cytology result which led to costly and unnecessary tests which carries additional risk and contributes to patient anxiety.

4.5 Limitations

There are several limitations to this study. While I did not identify any patients with UTUC that presented with NVH, it is plausible that patients with UTUC in the VH cohort might have initially presented with NVH before VH if screening for NVH was performed although this is not recommended by any consensus. In addition, all 18 patients with a diagnosis of UTUC had a CT urogram, of which 7 patients (38.9%) had a RBUS as well. It is plausible that UTUC might have been present amongst the 1,691 patients who only had a RBUS although we cannot confirm this given the pragmatic nature of this study.

While sonographers require a distended bladder to adequately evaluate the bladder for malignancy, this was not performed in all cases. Similarly, assessment of the urinary bladder was limited in some CTU scans where contrast did not opacify the bladder or where there was artefact due to metal prosthesis in the pelvis. To account for these suboptimal scans, we exclude these scans to determine the diagnostic accuracy of imaging in identifying bladder cancer. Additionally, I cannot determine the sensitivity of cystoscopy as we are unable to determine if tumours were missed in patients with a normal cystoscopy as these patients were discharged and did not have a repeat test.

With regards to urine cytology, there was variation in methods for urine collection and processing for cytopathological analysis due to the <multicentre nature of the study. The classification of positive cytological analysis may be different between cytopathologists. There was also no central review of cytology results. These results reflect the diagnostic performance of urinary cytology throughout the UK which will inform policy makers that routine urine cytology is not necessary in the haematuria setting. However, I acknowledge that urine cytology may test positive due to a cancer anticipatory effect (186). While I do not have long term follow-up

for patients where cytology was positive with a normal cystoscopy and imaging, these patients were followed-up until discharged from urology care.

4.6 Conclusions

My results suggest that CTU can safely be replaced with RBUS to image the upper tracts in addition to cystoscopy as part of investigations following a presentation of NVH. The risk of UTUC in patients with NVH is extremely low and RBUS can identify renal parenchymal cancers with a high sensitivity. Where renal calculi is suspected, a non-contrast CTKUB with RBUS or CTU is necessary. Cystoscopy remains the diagnostic test of choice to detect bladder cancer.

I report that there is no role for the routine use of urine cytology in the haematuria diagnostic pathway. Urine cytology will miss a significant number of muscle invasive bladder cancer and high risk non-muscle invasive disease. My results suggest that urine cytology should not be routinely performed as part of haematuria investigations. Until urinary biomarkers with a high diagnostic accuracy have been independently validated, cystoscopy and upper tract imaging will remain the cornerstone test for patients with haematuria.

CHAPTER 5 : SYSTEMATIC REVIEW OF NOVEL URINARY BIOMARKERS

5.1 Introduction

Cystoscopy remains the gold standard for the detection of bladder cancer in patients following a presentation of haematuria and in patients requiring surveillance for recurrent disease following resection of the initial tumour. However, cystoscopy is invasive and has a 5% risk of UTI (20). The requirement for life long surveillance in high risk patients have significant healthcare cost implications. Hence, there is an urgent need to develop a highly specific and sensitive urinary biomarker for the detection of bladder cancer.

Currently the US FDA has approved six urinary assays for clinical use; BTA stat (Polymedco), BTA TRAK (Polymedco), NMP22 (Matritech), NMP22 BladderCheck Test (Alere), uCyt (Scimedx) and UroVysion (Abbott Molecular). These tests report an overall sensitivity between 57-82% and specificity between 74-88% (131). Such suboptimal diagnostic performance has led to the recommendation that none of these assays are approved for use without cystoscopy although they may have a role to reduce the frequency of surveillance cystoscopy.

There has been considerable interest in the development of urinary biomarkers as evident by the large number of published reports. While many show promising results, few have been reproduced in subsequent independent validation studies. Traditional assays have been designed for single targets or small panel assays restrained by the technology and assay performance. More recently, next generation sequencing and advancements in bioinformatics has enabled a paradigm shift whereby biomarker panels comprising of multiple targets has been utilised using small quantities of input DNA.

In this chapter, I performed a systematic review comprising of a literature search between January 2013 to July 2017 to provide an update of urinary biomarkers for the detection of bladder cancer across the spectrum of protein, genomic, epigenetic and transcriptomic biomarkers. The purpose of this systematic review is to highlight promising biomarkers which may have clinical utility in the future. Results from this chapter has been previously published in the journal *Cancer Treatment Reviews* (187).

5.2 Material & methods

5.2.1 Literature search

A systematic search of the literature was performed using MEDLINE / PubMed to identify articles evaluating novel urine biomarkers for the detection of bladder cancer. A comprehensive literature search was performed between 1st January 2013 and 31st July 2017 using the following keywords and MeSH terms: (bladder cancer OR transitional cell carcinoma OR urothelial cell carcinoma) AND (detection OR diagnosis) AND urine AND (biomarker OR assay). The search protocol was registered in the PROSPERO database (CRD42016049918, Appendix A1).

5.2.2 Study selection

Articles selected were written in English and reported the diagnostic characteristics of novel urinary biomarkers for the detection of bladder cancer. Following screening of abstracts to exclude review articles, comments and letters to the editor or non-relevant articles, each manuscript was reviewed, and data was extracted and references searched for relevant missing manuscripts.

All studies required a minimum of ≥20 patients in both bladder cancer and control arm to be included and report both sensitivity and/ or specificity and / or receiver operating characteristics area under the curve (AUC). The presence of bladder cancer was defined as the presence of cancer at histopathological examination following transurethral resection of bladder cancer. Biomarkers were classified to protein, genomic, epigenetic, transcriptomic and combination ‘omic’ biomarkers.

All abstracts and full text were independently screened by two investigators. When there were disagreements, this was discussed with a third investigator and resolved by a consensus view. Cohort and cross-sectional studies were included.

5.2.3 Data extraction and quality assessment

Data was extracted from selected studies about type of biomarker used, assay used, study design, percentage of low grade cancer assayed, urine collection details and number of patients with bladder cancer and controls. When more than one patient cohort were described, the final validation patient group was used. Low grade tumours were defined according to EAU risk classification (25). A 2 X 2 table with number of true-positive, false-positive, true-negative, and false-negative results from published sample sizes was constructed to determine the sensitivity, specificity, PPV and NPV where available. AUC where reported was included. A second investigator confirmed data were extracted accurately. QUADAS-2 tool was used to assess risk of bias and concerns about applicability of studies (188).

5.3 Results

5.3.1 Characterization of studies

The PRISMA flowchart is shown in Figure 5.1. The database search identified 646 articles and after the addition of other relevant articles, a total of 656 abstracts were screened. Dual review of abstracts and titles excluded 377 studies which were not original research, not in English or unrelated articles. A further 164 studies were excluded after full text review as they did not meet the inclusion criteria leaving 115 articles which were included for analysis.

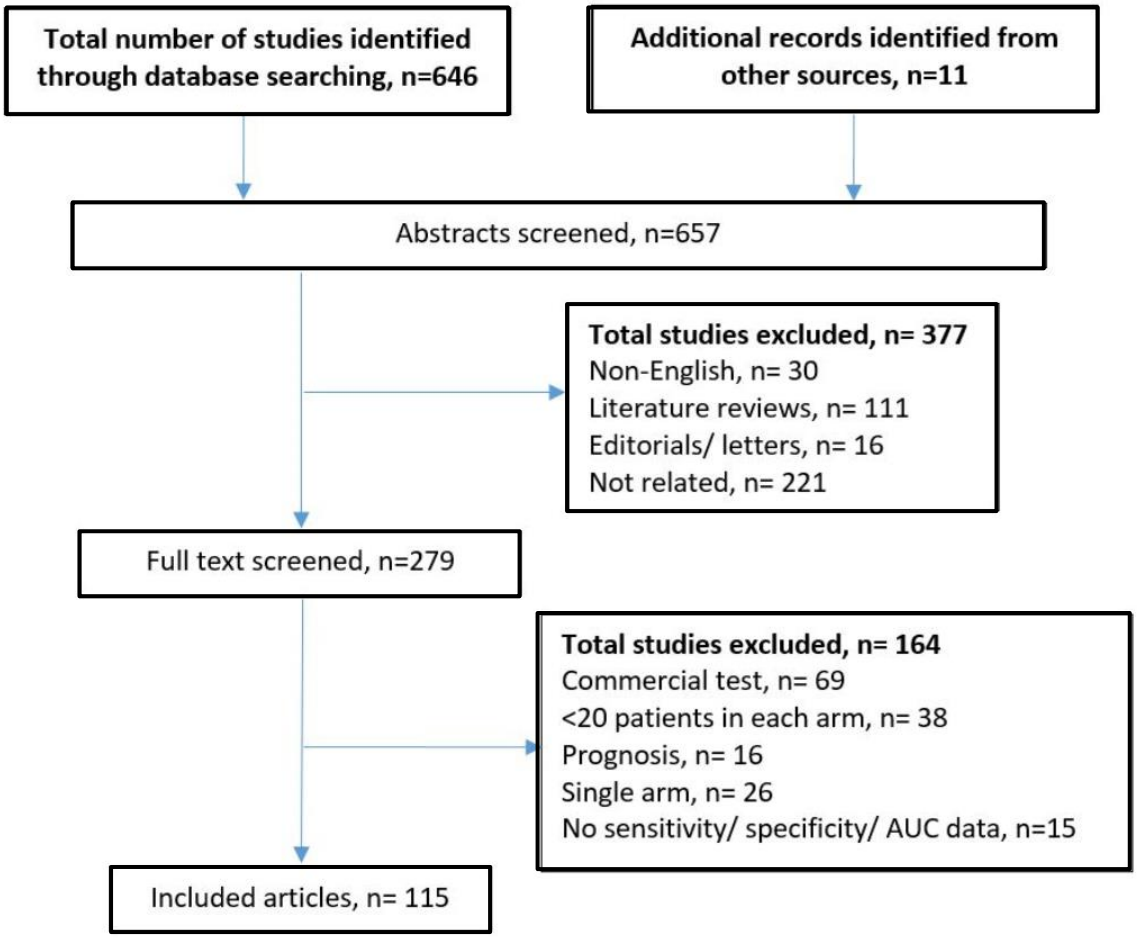
Articles were then classified to the following biomarkers: protein (n=59), genomic (n=7), epigenetic (n=19), transcriptomic (n=21) or combination of different 'omic' biomarkers (n=10). Twenty five protein (189-213), 1 genomic (214), 8 epigenetic (140, 215-221), 10 transcriptomic (222-231) and 6 combination of different 'omic' (232-237) biomarkers had a sensitivity and specificity ≥80%. Studies with a sensitivity and specificity of <80% are shown in Tables 5.3, 2.4, 2.6, 2.8, 2.10). Of the studies with a sensitivity and specificity ≥80%, most of these studies were designed as case control with selected groups comprising of urine from bladder cancer and control cases indicating selection bias (Table 5.1). Four were prospective observational studies, one of which incorporated sequential urine sampling with surveillance cystoscopy, although none had pre-planned statistical power calculations (220, 228, 229, 234). Twenty three studies had a low risk of bias in determining the characteristics of the index test according to the QUADAS-2 tool (140, 197, 199, 200, 202, 203, 205, 206, 209, 210, 212, 215, 217-221, 223, 228-230, 233, 234). Quality assessment using the QUADAS-2 tool for individual studies are summarized in Table 5.1.

Table 5.1: Overview of quality assessment according to the QUADAS-2 tool per study

Authors	Risk of bias				Concerns about applicability		
	Patient selection	Index test	Reference test	Flow and timing	Patient selection	Index test	Reference test
Protein biomarkers							
Li et al. 2016 (189)	High	High	Low	Low	Low	Low	Low
Abd El-Hakim et al. 2014 (191)	High	High	Low	Low	Low	Low	Low
Srivastava et al. 2013 (192)	High	High	Low	Low	Low	Low	Low
Choi et al. 2016 (190)	High	High	Low	Low	Low	Low	Low
Srivastava et al. 2016 (195)	High	High	Low	Low	Low	Low	Low
Zhou et al. 2016 (197)	High	Low	Low	Low	Low	Low	Low
Lorenzi et al. 2013 (193)	High	Unclear	Low	Low	Low	Low	Low
Li et al. 2014 (199)	High	Low	Low	Low	Low	Low	Low
Ebbing et al. 2014 (196)	High	High	Low	Low	Low	Low	Low
Attallah et al. 2015 (194)	High	High	Low	Low	Low	Low	Low
Shimada et al. 2016 (213)	High	Unclear	Low	Low	Low	Low	Low
Soukup et al. 2015 (198)	High	High	Low	Low	Low	Low	Low
Jamshidian et al. 2014 (200)	High	Low	Low	Low	Low	Low	Low
Kumar et al. 2015 (205)	High	Low	Low	Low	Low	Low	Low
Rosser et al. 2014 (206)	High	Low	Low	Low	Low	Low	Low
Goodison et al. 2016 (201)	High	High	Low	Low	Low	Low	Low
Shimizu et al. 2016 (202)	High	Low	Low	Low	Low	Low	Low
Chen et al. 2014 (203)	High	Low	Low	Low	Low	Low	Low
Rosser et al. 2014 (204)	High	Unclear	Low	Low	Low	Low	Low
Gok et al. 2016 (207)	High	Unclear	Low	Low	Low	Low	Low
Nakai et al. 2015 (208)	High	High	Low	Low	Low	Low	Low
Inoue et al 2014 (209)	High	Low	Low	Low	Low	Low	Low
Jin et al. 2014 (210)	High	Low	Low	Low	Low	Low	Low

Shen et al. 2015 (211)	High	High	Low	Low	Low	Low	Low
Aggio et al. 2016 (212)	High	Low	Low	Low	Low	Low	Low
Epigenetic biomarkers							
Zhang et al. 2014 (215)	High	Low	Low	Low	Low	Low	Low
Eissa et al. 2015 (216)	High	Unclear	Low	Low	Low	Low	Low
Mengual et al. 2013 (217)	High	Low	Low	Low	Low	Low	Low
Urquidi et al. 2016 (218)	High	Low	Low	Low	Low	Low	Low
Du et al. 2017 (219)	High	Low	Low	Low	Low	Low	Low
Su et al. 2014 (220)	Low	Low	Low	Low	Low	Low	Low
Wang et al. 2016 (221)	High	Low	Low	Low	Low	Low	Low
Feber et al. 2017 (140)	High	Low	Low	Low	Low	Low	Low
Transcriptomic biomarkers							
Ismail et al. 2016 (222)	High	High	Low	Low	Low	Low	Low
De Martino et al. 2015 (223)	High	Low	Low	Low	Low	Low	Low
Eissa et al. 2014 (224)	High	Unclear	Low	Low	Low	Low	Low
Eissa et al. 2014 (225)	High	Unclear	Low	Low	Low	Low	Low
Srivastava et al. 2014 (226)	High	Unclear	Low	Low	Low	Low	Low
Schmidt et al. 2016 (227)	High	High	Low	Low	Low	Low	Low
Ribal et al. 2016 (228)	Low	Low	Low	Low	Low	Low	Low
Mengual et al 2014 (229)	Low	Low	Low	Low	Low	Low	Low
Salomo et al. 2017 (230)	High	Low	Low	Low	Low	Low	Low
Eissa et al. 2015 (231)	High	Unclear	Low	Low	Low	Low	Low
Different combination 'omic' urinary biomarkers							
Van Kessel et al. 2016 (232)	High	High	Low	Low	Low	Low	Low
Van Kessel et al. 2016 (233)	High	Low	High	Low	Low	Low	Low
Dahmcke et al. 2016 (234)	Low	Low	Low	Low	Low	Low	Low
Eissa et al. 2013 (235)	High	Unclear	Low	Low	Low	Low	Low
Eissa et al. 2013 (236)	High	High	Low	Low	Low	Low	Low
Eissa et al. 2015 (237)	High	High	Low	Low	Low	Low	Low

Figure 5.1: Flow chart of studies identified, excluded and included in systematic review.



5.3.2 Protein biomarkers

Protein based biomarkers were the most commonly tested biomarker for the detection of bladder cancer, using either immunoassays such as ELISA (n=35) or spectrometry (n=9) for protein quantification. Multiple protein targets were tested in 14 studies using multiplex immunoassay platforms interrogating between 3-10 biomarkers (Table 5.2 & 5.3).

Fourteen tests which assessed an individual protein biomarker reporting a sensitivity and specificity ≥80% (189-197, 199, 200, 206, 209, 213) (Table 5.2). Of these, Orosomucoid 1 (ORM1), an acute phase transport protein, identified using mass spectrometry was quantified using enzyme-linked immunosorbent

assay (ELISA) of urine, with a sensitivity of 92%, specificity of 94% and an AUC of 0.965 (189). A separate study of 152 patients reported good diagnostic accuracy using the serine protease, HtrA1, and achieved a sensitivity of 93% and specificity of 96% (193).

Survivin, a protein which is implicated in the inhibition of apoptosis, has been investigated by a number of studies (191, 192, 238). Quantification of survivin using ELISA reports a sensitivity of 71-85% with a specificity of 81-95% (191, 192, 238). Soluble Fas was reported by two studies and showed varying sensitivity of 51% and 88% which suggest a lack of reproducibility (195, 239).

Amplified in breast cancer 1 (AIB1) which has been shown to promote cell proliferation via AKT pathway had a sensitivity and specificity of 80% and 86% respectively (240). Combining eukaryotic initiation factor 2 (EIF5A2) with nuclear matrix protein (NMP22) increased the sensitivity to 89%, specificity to 91% and reported an AUC of 0.898 (197). Other reports on single protein biomarkers include apurinic/apyrimidinic endonuclease 1/redox factor-1 (APE1/Ref-1), apolipoprotein A-I (Apo-A1), calprotectin, and NMP52 reported a sensitivity and specificity ranging from 82-94% and 80-93% respectively (190, 194, 196, 199).

Four studies reported the diagnostic ability of proteins cytokeratin 8 and 18 using the UBC Rapid point of care Omega 100 reader (241-244). Cytokeratin are constituents of intermediate filaments of epithelial cells. This point of care test requires three drops of urine, and the result from a photometric reader is available within 10 minutes. The sensitivity of the assay ranges from 30-87% with CIS patients having the highest sensitivity and a specificity of between 63- 91% and an AUC of up to 0.750 suggesting limited diagnostic performance. One study investigated the role of Ubiquilin 2 immunocytological staining reporting a

sensitivity and specificity of 88% and 98% respectively, although results for cytological based test were operator dependent (213).

A combination of urinary cytology, midkine (NEGF2) and gamma synuclein quantification using ELISA reported an AUC of 0.949 with a sensitivity and specificity of 91.8% and 97.5% respectively (206). The nonsulfated glycosaminoglycan hyaluronic acid (HA) quantified by ELISA reported a sensitivity and specificity of 88% and 82% increasing to 90% and 84% respectively when combined with hyaluronidase, a catalytic enzyme that degrades HA (200). Another 5-panel biomarker using gamma synuclein with Coronin-1A, Apolipoprotein A4, Semenogelin-2 and DJ-1 / PARK7 compared ELISA to Western blot (205). Western blot achieved higher sensitivity (93.9% vs 79.2%) and a similar specificity (97% vs 100%) compared to ELISA in pTa/ pT1 cancers (205). However, western blot for protein quantification would not be practical in a large scale setting. Rosser et al. reported an AUC of 0.948 using a multiplex ELISA system when combining three biomarkers: Interleukin 8 (Il-8), Matrix metallopeptidase 9 (MMP9) and vascular endothelial growth factor A (VEGFA) (206). However, further studies incorporating the same three biomarkers and with the addition of between a further 4-7 markers have yielded an AUC of between 0.878-0.926 on validation studies (201-204, 245).

Six studies utilising spectroscopy or chromatography to determine a metabolic signature or a molecular compound had a sensitivity and specificity of ≥80% (207-210) (211, 212). Several of these assays achieve sensitivity and specificities of ≥90% but have not been externally validated (209, 210, 212).

Table 5.2: Study characteristics and diagnostic accuracy of urinary protein biomarkers for the diagnosis of bladder cancer with sensitivity and specificity ≥80%.

Title	Type of marker	Marker	Test platform	Study design	Urine collection	Low Grade (%)	Tumour arm	Control arm	Sensitivity	Specificity	PPV	NPV	AUC
Li et al. 2016 (189)	Transport protein	ORM1	ELISA	Case control	20 ml morning void	35	112	53	92	94			0.965
Abd El-Hakim et al. 2014 (191)	Inhibitor of apoptosis protein	Survivin	ELISA	Case control	Not specified	25	40	20	85	95	94	86	0.950
Srivastava et al. 2013 (192)	Inhibitor of apoptosis protein	Survivin	ELISA	Case control	50 ml void	41	117	74	83	81			0.881
Choi et al. 2016 (190)	DNA repair protein	APE1/Ref-1	ELISA	Case control	Not specified	58	169	108	82	80	86	73	0.830
Srivastava et al. 2016 (195)	cell-surface receptor for apoptosis	Soluble FAS	ELISA	Case control	50 ml void	25	117	74	88	89			0.912
Zhou et al. 2016 (197)	Transcription coactivator (AIB1), transcription kinase (EIF4A2),	AIB1	ELISA	Case control	50 ml midstream fist void	42	134	76	80	86	91	71	0.827
		Combination of AIB1 + EIF5A2 + NMP22							89	91	94	82	0.898
Lorenzi et al. 2013 (193)	Serine protease	HtrA1	ELISA	Case control	First void	Not specified	68	84	93	96	95	93	0.984
Li et al. 2014 (199)	HDL related protein	Apo-A1	ELISA	Case control	50 ml midstream first void	Not specified	223	156	89	85			0.948
		Apo-A1 + cytology							94	84			
Ebbing et al. 2014 (196)	Inflammation related protein	calprotectin	ELISA	Case control	10 ml void	54	46	40	80	93	93	80	0.88
Attallah et al. 2015 (194)	Nuclear matrix protein	NMP52	ELISA	Case control	Not specified	19	62	94	94	80			0.91
Shimada et al. 2016 (213)	Regulatory protein	Ubiquitin 2	Immunocytology	Case control	Not specified	29	102	143	88	99	98	93	
Soukup et al. 2015 (198)	Heparin binding growth factor (midkine), peripheral nervous system protein (gamma synclein)	cytology+ midkine + gamma synuclein	ELISA	Case control	Second morning void	27	70	49	92	98	98	89	0.949
Jamshidian et al. 2014 (200)	Glycoaminoglycan (hyaluronic acid), Hydrolytic enzyme (hyaluronidase)	Hyaluronidase	ELISA	Case control	Not specified	47	97	97	88	82			
		Hyaluronic acid							83	90			
		Hyaluronidase + hyaluronic acid							90	84			
Kumar et al. 2015 (205)	Actin binding protein (Coronin-1A), Apolipoprotein (Apo-A4),Gell matrix protein (Semenogelin-2), transmembrane (type I) heparan sulfate proteoglycan	DJ-1/PARK7	ELISA & Western	Case control	20 ml void	All pTa/pT1	173	66	83-96	100	100	71-91	
		Coronin-1A + Apo-A4 + Semenogelin-2 + Gamma synuclein + DJ-1/PARK7							79 (ELISA)/ 94 (western)	100 (ELISA)/ 97 (western)			0.920 (ELISA)/ 0.980 (western)

	(Gamma synuclein), Peptidase (PARK7/ DJ-1)												
Rosser et al. 2014 (206)	Chemokine (IL8), Protease (MMP9), Growth factor (VEGF-A)	IL-8	ELISA	Case control	50 ml void	45	31	42	90	86	82	92	0.907
		IL8+ MMP9 + VEGFA							93	81	78	94	0.948
Goodison et al. 2016 (201)	Chemokine (IL-8), Protease (MMP9, MMP10), Inhibitor of serine proteases (SERPINA1), Hydrolyzes cellular RNA and promotes angiogenesis (Angiogenin), Growth factor (VEGF-A), zinc metalloenzymes (Carbonic anhydrase 9), Apolipoprotein (APOE), Serine protease inhibitor (type I) heparan sulfate proteoglycan (SDC1)	10 biomarker panel: IL8, MMP9 & 10, SERPINA1, Angiogenin, VEGF-A, Carbonic anhydrase 9, APOE, PAI-1, SDC1 Matrix metallopeptidase 9 (MMP9)	MULTI-ARRAY technology-custom multiplex immunoassay	Retrospective case control	Not specified	38	211	67	85	81	93	63	0.893
Shimizu et al. 2016 (202)			multiplex array compared to ELISA	Case control	Not specified	17	100	100	85	81	82	84	0.926
Chen et al. 2014 (203)			ELISA	Case control	>3 ml void	32	183	137	79	79	73	84	0.848
Rosser et al. 2014 (204)			ELISA	Case control	50 ml void	57	53	72	79	88	82	85	0.904
Gok et al. 2016 (207)	Molecule signature	Reflection mode: Spectral range- 1500-1340, 1100-900, 900-800	Infrared spectroscopy	Case control	10 ml bladder wash	Not specified	40	21	82	81	90	81	
Nakai et al. 2015 (208)	porphyrin	difference between ALA treated and ALA untreated samples at 635 nm	spectrophotometry	Case control	150 ml void	46	61	50	82	80			0.840
Inoue et al 2014 (209)	porphyrin	uroporphyrin I (UPI)	Florescence spectroscopy	Case control	15 ml void	n/a	66	20	100	96			0.994
		coproporphyrin I (CPI)							100	92			0.978
		coproporphyrin III (CPIII)							80	82			0.828
		total porphyrins							80	94			0.827
Jin et al. 2014 (210)	Metaolic signature	OPLAS-DA model: 12 peaks corresponding to. succinate, pyruvate, oxoglutarate, carnitine, phosphoenolpyruvate, trimethyllysine, melatonin, isavalsrylcarnitine, glytarylcarnitine, octenoylcarnitine, decanoylcarnitine, acetyl-coA	Mass spectroscopy	Case control	Morning void	23	138	121	91	93			0.937
Shen et al. 2015 (211)	Metabolic signature	MixModel1: GlyCysAlaLys, Inosinic acid, Trehalose, Nicotinuric acid, Asp Asp Gly Trp, Ureidosuccinic acid	Mass spectroscopy	Case control	Morning void	Not specified	23	21	91	81			0.934
Aggio et al. 2016 (212)	Metabolic signature	Principal component analysis	gas chromatography	Case control	0.75 ml of morning void	Not specified	24	73	96	100			0.990

AIB1: amplified in breast cancer 1; APE1/Ref-1: apurinic/apyrimidinic endonuclease 1/redox factor-1; I Apo-A1: apolipoprotein A1; Apo-A4: apolipoprotein A4; Apo-E: Apolipoprotein E; EIF5A2: eukaryotic initiation factor 2; NPV: negative predictive value; NMP22: nuclear matrix protein 22; NMP52: nuclear matrix protein 52; ORM1: orosomucoid 1; SDC1: Syndecan; IL8: Interleukin 8, MMP9: Matrix metallopeptidase 9; MMP10: Matrix metallopeptidase 10; VEGF-A: Vascular endothelial growth factor A; PPV: Positive predictive value; PAI-1: Plasminogen activator inhibitor-1

Table 5.3: Study characteristics and diagnostic accuracy of urinary protein biomarkers for the diagnosis of bladder cancer with sensitivity and/ or specificity <80%.

Title	Type of marker	Marker	Test platform	Study design	Urine collection	Low Grade (%)	Tumour arm	Control arm	Sensitivity	Specificity	PPV	NPV	AUC
Vu Van et al. 2016 (246)	Heparin binding growth factor	Midkine	ELISA	Prospective case control	50 ml random non-first void	18	92	70	70	77	81	65	0.759
Li et al. 2013 (238)	Inhibitor of apoptosis protein	Survivin	ELISA	Prospective case control	10 ml midstream void	27	102	102	71	89			0.849
Liu et al. 2016 (247)	Peripheral nervous system protein	Gamma-synuclein	ELISA	Blinded prospective case control	Not specified		141	135	68	97.	97	72	0.903
Yang et al. 2013 (239)	Cell-surface receptor for apoptosis	soluble FAS	ELISA	Prospective case control	10-20 ml void	44	128	88	51	86	65	78	0.732
Yasar et al. 2016 (248)	Inflammatory glycoprotein	YKL-40	ELISA	Case control	20 ml void	43	67	65	55	46			0.515
Arikan et al. et al. 2015 (249)	Epidermal growth factor receptor	Human epidermal growth factor receptor 2 (HER2/neu)/ creatinine ratio	ELISA	Case control	Not specified	57	44	40	32	88	90	74	
Kamada et al. 2015 (250)	Extracellular matrix protein	Laminin-332/ creatinine	ELISA	Case control	Not specified		39	61	97	46	54	97	0.783
Nakashima et al. 2015 (251)	Cytokine	CXCL1/ creatinine	ELISA	Case control	Not specified	34	175	30	49	95			0.840
Zhu et al. 2016 (252)	Cytokine	CXCL5/ creatinine	ELISA	Case control	Not specified	27	92	80	80	61			0.695
Morgan et al. 2013 (253)	Homeobox-containing gene	Engrailed-2 (EN2)	ELISA	Case control	Not specified	24	466	55	82	75			0.844
Laloglu et al. 2016 (254)	Proteoglycans	Endocan/ endothelial cell-specific molecule-1	ELISA	Case control	Not specified	Not specified	50	100	62	71			
Candido et al. 2016 (255)	lipocalin superfamily found in activated neutrophils	Neutrophil gelatinase-associated lipocalin (NGAL)	ELISA	Retrospective case control	50 ml first void	5% well-moderately	89	119	61	61	53	67	
	Protease	matrix metalloproteinase (MMP)-9							65	66	59	72	0.700
		NGAL/MMP-9 complex							65	67	60	72	0.700
	Peptide hormone	angiotensin	ELISA	Case control	50-100 ml void	33	50	40	66	75	77	64	0.803

Shabeyek et al. 2014 (256)	Extracellular chaperone protein	clusterin							70	83	83	69	0.817
		Angiotensin + clusterin							82	68	76	75	
		Angiotensin + clusterin+ cytology							88	55	71	79	
Badr et al. 2013 (257)	transcriptional regulator of cellular and developmental response to hypoxia	Hypoxia-inducible factor 1-alpha (HIF 1A)	ELISA	Case control	Voided urine	NA	39	30	82	63			
		HIF 1A + cytology							54	93			
Miyake et al. 2013 (258)	Cytokine	Chemokine (C-C motif) ligand 18 (CCL18)	ELISA	Case control	50 ml void	37	102	206	70	68	53	82	0.768
	Protease inhibitor	Alpha-1 antitrypsin (A1AT)							71	72	55	83	0.775
Rosser et al. 2013 (245)	Protein	7 biomarkers: IL-8, MMP-9 and 10, PAI-1, VEGF, ANG, APOE	ELISA	Case control	50 ml void	38	102	206	74	90	79	87	0.878
	IL-8- chemokine; PAI-1- serine protease inhibitor; MMP-9-protease	Interleukin 8 (IL8), Plasminogen activator inhibitor-1 (PAI-1), Matrix metallopeptidase 9 (MMP-9)							79	84	70	89	0.861
Shimwell et al. 2013(259) .	Heparin binding growth factor	midkine	ELISA	Prospective case control	Voided urine	22	562	80					Ta: 0.574, T1: 0.708, T2+ 0.851
	Serine proteinase inhibitor	Hepatocyte growth factor activator inhibitor type 1 (HAI-1)											Ta: 0.566, T1: 0.748, T2+: 0.886
Styrke et al. 2016 (241)	Keratin protein in intracytoplastic cytoskeleton of ephthelial tissue	Cytokeratins 8 & 18	UBC Rapid-point of care Omega 100 POC reader	Case control	4 drops of urine	31	137	133	71	61	71	61	0.702
						24	94	101	82	63			
Ecke et al. 2017 (242)				Case control	4 drops of urine	26	87	22	LGNMIBC :30.4%. HGNMIBC: 71.4%, MIBC:60 %	91			0.750
Ritter et al. 2014 (243)				Prospective study on patients suspicious	4 drops of urine	45	61	137	61	70	47	79	0.680

				for bladder cancer									
Ecke et al. 2015 (244)				Case control	4 drops of urine	49	92	33	46-68	91			0.733
Zhou et al. 2017 (260)	Metabolic signature	5-hydroxyvaleric acid, cholesterol, 3-phosphoglyceric acid and glycolic acid	Mass spectrom etry	Case control	100 µl urine	46	59	37	78	70			0.804
Frantzi et al. 2016 (261)	Metabolic signature	116 peptide- detection primary, 106 peptide-detect recurrence	Mass spectrom etry	Prospective study on patients suspicious for bladder cancer	Not specified	17.3	168	102	91	68	81	83	0.880
Koslinski et al. 2016 (262)	Compound	Pterin acid	Chromato graph	Case control	400 µl urine	Not specified	46	35	2	97			0.560
		Xanthopterin							2	93			0.620
		Isoxanthopterin							30	78			0.630
		Biopterin							2	95			0.570
		Pterin							33	67			0.560
		Neopterin							2	97			0.610
Wittlmann et al. 2014 (263)	Metabolic signature	6 metabolite assay: palmitoyl sphingomyelin, lactate, gluconate, adenosine, 2-methylbutyrylglycine, guanidinoacetate	Mass spectrom etry	Prospective case control	Not specified	34	29	79					0.780
Davis et al. 2016 (264)	Immunocytology	Celldetect	Histoche mical staining	Blinded case control	50 ml void	42.7	96	121	84	70			
McNeil et al. 2014 (265)	Cell surface tyrosine kinase receptor	soluble Met	elctroche milumines cent immunoas say	Case control	Not specified	Not specified	183	83	61	76	85	47	0.701
Khadjavi et al. 2015 (266)	Phosphorylated protein	tyrosine-phosphorylated proteins (UPY)	chemilumi nescence reader	Case control	10-50 ml void	47	92	170	80	79	67	88	0.920
Mohammed et al. 2013 (267)	Matrix metalloproteinas es	MMP-2	Zymograp hic analysis	Case control	Voided urine	Grade 1/2: 65%	66	100	55	100	100	77	
		MMP-9							62	100	100	80	
		MMP9/ NGAL							61	100	100	79	
		MMP-9 dimer							53	100	81	76	
		MMP-9/ TIMP-1							12	100	65	63	
		ADAMTS							26	100	70	67	
		Total							64	100	100	81	
Sankiewicz et al. 2016 (268)	Mucin-type transmembrane protein	podoplanin	Surface Plasmon Resonanc	Case control	Not specified	43	82	27	72	44	80	34	0.660

			e Imaging biosensor										
Chen et al. 2013 (269)	Apolipoprotein	APOA1	Multiplex immunoassay-MILLIPEX MAP human apolipoprotein panel kit	Case control	12.5 ml morning void	32	63	48					0.875
		APOA2											0.864
		APOB											0.739
		APOC2											0.838
		APOC3											0.835
		APOE											0.745
Ardelt al. 2013 (270)	Actin binding protein	LASP-1	Western blot	Case control	10 ml void	37	63	69	83	85	83	81	0.700
Blanca et al. 2016 (271)	Tyrosine kinase receptor	Fibroblast growth factor receptor 3 (FGFR3)	Western blot	Case control	Not specified	52	110	211	42	98	94	77	0.702
	Cell cycle kinase	Cyclin D3							51	90	74	78	0.707
		Combination of FGFR3 +Cyclin D3							73	90	79	86	0.810

AIB1: amplified in breast cancer 1; APE1/Ref-1: apurinic/apyrimidinic endonuclease 1/redox factor-1; I Apo-A1: apolipoprotein A; Apo-A4: apolipoprotein A4; Apo-E: Apolipoprotein E; EIF5A2: eukaryotic initiation factor 2; NMP22: nuclear matrix protein 22; NMP52: nuclear matrix protein 52; ORM1: orosomucoid 1; SDC1: Syndecan; IL8: Interleukin 8, MMP9: Matrix metallopeptidase 9; MMP10: Matrix metallopeptidase 10; VEGF-A: Vascular endothelial growth factor A; PAI-1: Plasminogen activator inhibitor-1

5.3.3 Genomic biomarkers

Seven studies investigated the role of genomic biomarkers for the detection of bladder cancer. Four were based on analysis of mutations and included in Table 5.4. Mutations in *TERT* (Telomerase reverse transcriptase) promoter represent the most common bladder cancer mutation present in >70% of all bladder cancers (272). One study by Descotes and colleagues reported a sensitivity and specificity of 81% and 90% respectively for *TERT* although others have reported a lower sensitivity of 62% (214, 272, 273). *TERT* mutation was also associated with a >5-fold increase relative risk of recurrence (p=0.0004) (214).

FGFR3 achieved a sensitivity of 39% as a standalone test for bladder cancer (274). *FGFR3* mutation is more common in low grade disease (p=0.02) and significantly associated with shorter time to recurrence (45% mutant vs 27% wild type, p=0.02) (274, 275). Other mutations such as *TP53*, *PIK3CA* and *RAS* have reported limited performance because of the low frequency of mutations and variability of genomic alterations between individual tumours. Sensitivity for the detection of *TP53* of 12-13%, *PIK3CA* 13-14% and *RAS* 4.8% have been reported (273, 275). The diagnostic performance of the combination of *FGFR3* and *TERT* with *PIK3CA*, *RAS* and *TP53* improved bladder cancer detection but only achieved a sensitivity of 73% (273). Of note, it has been demonstrated that following complete resection of tumour, 20.7% of patients will continue to test positive for *FGFR3* and *TERT* mutation despite no cystoscopic detectable tumour in patients followed up for 3 years (275). In addition to targeted mutation analysis, the quantitative cell-free DNA analysis has been explored as a marker for the presence of bladder cancer as well as analysis of the integrity of cell-free DNA.

To date studies are preliminary and report limited diagnostic performance with an AUC of 0.725-0.834 (276, 277).

Table 5.4: Study characteristics and diagnostic accuracy of urinary genomic biomarkers for the diagnosis of bladder cancer with sensitivity and/ or specificity <80%.

Title	Type of marker	Marker	Test platform	Study design	Urine collection	Low Grade	Cancer arm	Control arm	Sensitivity	Specificity	PPV	NPV	AUC
Ward et al. 2016 (273)	Catalytic subunit of telomerase (TERT), fibroblast growth factor receptor (FGFR3), nuclear receptor (RXRA), tumour suppressor gene (TP53), Intrcellular signal transducer enzymes (PIK3CA)	TERT, FGFR3, RXRA, TP53, PIK3CA	Multiplex PCR & NGS	Retrospective case control	20-50 ml voided	Not specified	120	20	70	97			
Couffignal et al. 2015 (274)	Fibroblast growth factor receptor	4 FGFR3 mutations: R248C, S249C, G372C, and Y375C	PCR	Prospective cohort	50-100 ml voided	58	191		39				
							74 patients with FGFR3 mutation followed up for 2 years with 33 recurrences		73	87	55	94	
Brisuda et al. 2016 (276)	Cell free DNA	Quantity of cell free DNA	RT-pPCR	Cohort	2nd morning void of 50 ml	44	66	34	43	91	90	45	0.725
Cussenot et al. 2013 (278)	DNA	BCA-1- 341	Oligo-CGH-array	Case control	Not specified	30	39	95	95	51	45	96	
Casadio et al. 2013 (277)	UCF DNA and cytology	UCF DNA integrity	RT-qPCR	Case control	First morning void	G1/2- 41	51	32	73	84			0.834
		UCF DNA integrity+ cytology							81	77			
Critelli et al. 2016 (275)	Catalytic subunit of telomerase (TERT), fibroblast growth factor receptor (FGFR3), Intrcellular signal transducer enzymes (PIK3CA), cellular transduction protein (RAS)	FGFR3	RT-qPCR	Primary tumour	10-100 ml void	30	230		42				
		TERT							52				
		TERT + FGFR3							67				
		TERT, FGFR3, PIK3CA, and RAS							69				
		TERT, FGFR3, PIK3CA, and RAS		Prospective cohort		Not specified	35 recurrences from 81 patients followed up for 3 years		77				

BCA-1: Bacterial artificial chromosome 1; FGFR3: fibroblast growth factor receptor 3; PIK3CA: phosphatidylinositol 3-kinase; RXRA: Retinoid X receptor alpha; TERT: Telomerase reverse transcriptase

5.3.4 Epigenetic biomarkers

Twelve studies reported the diagnostic performance of microRNA (miRNA) and 8 studies investigated the role of DNA methylation as a biomarker for the detection of bladder cancer (Table 5.5 & 5.6). No studies investigated the role of histone modifications. Overall, single target epigenetic biomarkers have a poor diagnostic performance, and epigenetic biomarker panels with a sensitivity and specificity of ≥80% are set out in Table 5.5. Biomarkers with a sensitivity and specificity <80% are shown in Table 5.6. Biomarker panels include between 2-150 targets to determine the presence of bladder cancer.

Of the miRNA panels, four have a sensitivity and specificity of ≥80% (Table 5.5) and employed miRNA arrays or next generation sequencing (NGS) to identify targets (215, 217-219). MiRNA was then quantified by real-time quantitative PCR (qPCR) (215-217, 219). MiRNA-125b was used in two diagnostic panels although its sensitivity and specificity as a single biomarker varies between 59-85 and 76-96% respectively (215, 279). The combination of two miRNAs, miRNA-99a and miRNA-125b, had a sensitivity and specificity of 87% and 81% respectively (215). Using multivariable modelling, Urquidi and colleagues, determined the diagnostic ability of the top 10, 15, 20 and 25 gene targets with the best bladder cancer diagnostic performance using a LASSO approach to model the performance of each biomarker (218). Their results suggest that incorporating increasing number of biomarkers can increase both sensitivity and specificity with marginal gains with each increase.

Only three of the 8 DNA methylation studies reported sensitivity and specificity ≥80% (Table 5.5). All studies included ≥3 DNA methylation targets and all report an AUC of >0.900. Methylation status was determined by quantitative methylation

specific PCR (qMS-PCR) (221), pyrosequencing (220) and next generation sequencing (140). Su and colleagues interrogated three methylated targets and deduced that the combination of *SOX1, IRAK3*, *L1-MET* methylation had sensitivity and specificity of 80% and 97% respectively (220). The three-target methylation panel of *POU4F2* + *PCDH17* + *GDF15* showed sensitivity and specificity of 91% and 88% respectively (221). Feber and colleagues derived a methylation signature of 150 loci incorporating a machine learning algorithm (140). The assay, UroMark, uses a targeted bisulphite sequencing approach and was validated with two independent sets of urine samples comprising of bladder cancer and control samples reporting a sensitivity of 98%, specificity of 97% and AUC of 0.970 (140).

Table 5.5: Study characteristics and diagnostic accuracy of urinary epigenetic for the diagnosis of bladder cancer with sensitivity and specificity ≥80%.

Title	Type of marker	Marker	Test platform	Study design	Urine collection	Low Grade (%)	Tumour arm	Control arm	Sensitivity	Specificity	PPV	NPV	AUC
Zhang et al. 2014 (215)	miRNA	miR-99a +miR-125b	RT-qPCR	Case control	Not specified. Urine supernatant	30	50	21	87	81	92	71	0.876
Eissa et al. 2015 (216)	miRNA	MiR-96+ cytology	RT-qPCR	Case control	30-60 ml void	G1/2=73	94	60	80	87	86	80	
Mengual et al. 2013 (217)	miRNA	6 miRNAs: miR-187 + miR-18a + miR-25 + miR-142-3p + miR-140-5p + miR-204	RT-qPCR	Case control	Not specified	38	151	126	85	87	88	83	0.921
Urquidi et al. 2016 (218)	miRNA	25 panel	RT-qPCR	Case control	30-50 ml midstream void	16	61	60	87	100			0.982
		10 panel							84	87			0.902
Du et al. 2017(219)	Cell free microRNA	7 cell-free miRNA: miR-7-5p, miR-22-3p, miR-29a-3p, miR-126-5p, miR-200a-3p, miR-375, and miR-423-5p	RT-qPCR	Case control	15 ml midstream urine. Urine supernatant	38	120	120	85	87			0.916
Su et al. 2014 (220)	DNA methylation	*SOX1 + IRAK3 + L1-MET*	Pyrosequencing	Prospective cohort	50 ml void/ bladder wash	41	34 recurrences from 90 patients between 5-89 months follow up		89	97			0.950
Wang et al. 2016 (221)	DNA methylation	*POU4F2*	qMS-PCR	Case control	Morning void	Not specified	72	92	91	92	88	94	0.921
		TCF21							86	82	76	90	0.910
		POU4F2 + EOMES							88	91	86	92	0.930
		POU4F2 + PCDH17							91	93	90	94	0.923
		POU4F2 + PCDH17 + GDF15							91	88	83	94	0.914
Feber et al. 2017 (140)	DNA methylation	150 CpG	RainDance microdroplet PCR, NGS	Case control	Voided urine	38	107	167	98	97		97	0.970

EOMES: Eomesodermin; GDF15: Growth/differentiation factor 15; IRAK3: Interleukin 1 Receptor Associated Kinase 3; L1-MET: Line 1 MET; NPV: negative predictive value; PPV: positive predictive value; PCDH17: Protocadherin-17; POU4F2: POU Class 4 Homeobox 2; TCF21: Transcription factor 21

Table 5.6: Study characteristics and diagnostic accuracy of urinary epigenetic biomarkers for the diagnosis of bladder cancer with sensitivity and/ or specificity <80%.

Title	Type of marker	Marker	Test platform	Study design	Urine collection	Low Grade (%)	Tumour arm	Control arm	Sensitivity	Specificity	PPV	NPV	AUC
Zhang et al. 2016 (280)	microRNA	miR-155	RT-qPCR	Case control	Morning void		162	162	80	85			0.585
Wang et al. 2015 (281)	Cell free microRNA	Cell-free microRNA-214	RT-qPCR	Case control	not specified	23	192	169	91	66	70	89	0.838
Zhou et al. 2014 (282)	microRNA	miR-106b	RT-qPCR	Case control	not specified	43	112	78	77	72			0.802
Shimizu et al. 2013 (283)	Methylated microRNA	miR-137	Bisulfite pyrosequencing	Case control	10 ml void	0	34	20	79	64			0.816
		niR124-2							79	91			0.866
		miR124-3							59	100			0.901
		miR9-3							77	73			0.797
		One methyl							94	64			
		2 methyl							82	91			
		3 methyl							71	91			
		4 methyl							41	100			
		Combination											0.910
Pospisilova et al. 2016 (279)	microRNA	miR-125b	RT-qPCR	Case control	50 ml void	56	27	23	59	96			0.801
		miR-204							54	100			0.771
		miR-99a							74	83			0.738
		miR-30b							67	83			0.760
		miR-532-3p							59	87			0.718
Sapre et al. 2016 (284)	microRNA	6X mRNA: miR16, miR200c, miR205, miR21, miR221 and miR34a	RT-qPCR, NGS	Case control	0.5 ml void	40	25	25	88	48	63	75	0.740
Hayashi et al. 2014 (285)	DNA methylation	*VGF*	RT-qPCR	Case control	Not specified	34	20	20	40	95	89	61	
Abern et al. 2014 (286)	DNA methylation	*TWIST1+NID2*	MS-qPCR	Prospective case control	30 ml void	50	24	87	75	71	42	92	0.730
Fantony et al. 2015 (287)	DNA methylation	*TWIST1 + NID2*	MS-qPCR	Prospective cross sectional	30 ml void	58	52	145	58-67	61-69	36-38	83-85	0.657
Fantony et al. 2017 (288)	DNA methylation: NID2, TWIST1	*NID2 + TWIST1*	MS-qPCR	Prospective case control	50 ml void	42	51	121					0.669
		NID2 + TWIST1 + suspicious/ atypical cytology positive											0.773
Garcia-Baquero et al. 2013 (289)	DNA methylation tumour suppressor genes: PRDM2, HLTF, ID4, DLC1, BNIP3, H2AFX, CACNA1G, TGIF, CACNA1A, CCND2,	*PRDM2*	Methylation Specific Multiplex Ligation Dependent Probe Amplification	Prospective case control	not specified	46	100	28	32	71	80	23	0.565
		RUNX3							37	82	88	27	0.655
		RARB							16	86	76	22	0.521
		HLTF-1							13	89	81	22	0.465
		HLTF-2							11	93	85	23	0.511
		SCGB3A1-1							42	71	82	23	0.543

	SCGB3A1, BNIP3, ID4, RUNX3	*SCGB3A1-2*						13	93	87	25	0.533
		ID4-1						28	71	76	23	0.539
		ID4-2						17	82	77	21	0.535
		TWIST1						23	82	18	22	0.538
		SFRP4-1						12	89	80	23	0.532
		SFRP4-2						12	93	86	23	0.562
		DLC1-1						22	82	82	24	0.533
		DLC1-2						18	93	90	24	0.594
		SFRP5-1						19	93	91	22	0.578
		SFRP5-2						10	89	77	21	0.556
		BNIP3						40	57	77	21	0.532
		H2AFX-1						19	79	76	21	0.469
		H2AFX-2						8	96	89	23	0.493
		CCND2-1						24	93	92	26	0.598
		CCND2-2						47	64	84	25	0.544
		CACNA1G						17	93	90	24	0.608
		TGIF						7	86	64	21	0.48
		BCL2						10	89	77	22	0.508
		CACNA1A						16	93	89	24	0.63
		TIMP3-1						12	93	86	23	0.546
		TIMP3-2						11	89	79	22	0.544

BCL 2: B cell lymphoma 2; CCND2: Cyclin D2; DLC1: Deleted in liver cancer 1; ID4: inhibitor of DNA binding protein 4; H2AFX-1: Histone 2A family member X; NID: Nidogen 2; RARB: Retinoic Acid Receptor Beta; RUNX3: Runt-related transcription factor 3; SCGB3A1-1: Secretoglobulin 3A1-1; SCGB3A1-2: Secretoglobulin 3A1-2; SFRP: Secreted frizzled-related protein; TGIF: Transforming growth factor- beta induced factor; TIMP3: Metalloproteinase inhibitor 3; VGF: vascular growth factor

5.3.5 Transcriptomic biomarkers

All studies used reverse transcriptase PCR (RT-PCR) to determine expression of target genes (Table 5.7 & 5.8). Four studies reported single target gene expression (222-224, 231) and four studies combined transcriptomic markers with urine cytology (225, 226, 230, 231) to achieve a sensitivity and specificity of ≥80% (Table 5.7). Of the four studies reporting a single biomarker, sensitivity ranges from 45-92%, specificity of between 65-96% and AUC of 0.741- 0.966. Studies reporting combination biomarkers achieved a sensitivity of 36-97%, specificity of 82-100% and an AUC of 0.860-0.949.

S100A4, carbonic anhydrase IX (CAIX) and hepatoma upregulated protein RNA (HURP) and long non-coding RNA urothelial carcinoma associated-1 (lncRNA-UCA1) represent single biomarker targets which have sensitivity and specificity of ≥80% (222-224, 231). De Martino and colleagues quantified CAIX in paired tumour and urine and validated their results in an independent cohort comprising 155 urine samples reporting a sensitivity, specificity and AUC of 81%, 96% and 0.883 respectively (223). Analysing six cytoplasmic calcium binding protein, S100A4 had the highest diagnostic accuracy with sensitivity of 90%, specificity of 92% and AUC of 0.978 (222).

Eissa and colleagues used gold nanoparticle based RT-PCR and reported a sensitivity of 89% and specificity of 94% for the presence of HURP (224). The technology performed better than conventional HURP RT-PCR, suggesting significant variation in results from different platforms (225). Another novel hybridization assay, nanoparticle RT-PCR of long non-coding RNA urothelial carcinoma associated-1 (lncRNA-UCA1) reported sensitivity and specificity of ≥90% and AUC of 0.966 (231). UCA1 has been implicated in bladder cancer

progression through PI3K-AKT dependent pathways and the development of cisplatin resistance via Wnt signalling (290, 291). However, conventional RT-PCR of lncRNA-UCA1 has not reproduced these results (292).

Cytokeratin 20 (CK20) was used as part of two multiplex assays (227, 230). In contrast to CK8 and 18, CK20 is expressed on urothelium but not epithelial cells, and has a reported diagnostic sensitivity, specificity and AUC of 76-85%, 86% and 0.820-0.870 respectively (227, 230). CK20 overexpression in combination with p53 and Ki-67 have been shown by immunohistochemistry to suggest urothelial dysplasia (293). The combination of cytology with CK20 has a sensitivity and specificity of ≥90% which has a higher diagnostic accuracy compared to other combinations such as Ki-67 with survivin, Ki-67 with CK20 and survivin with CK20 (227). When CK20 is used in combination with insulin like growth factor (IGF2), the sensitivity and specificity increased to 90% and 84% respectively (230).

The most promising transcriptomic panel that has been validated and tested in a prospective observational study is based on a combination of two genes IGF2 and Melanoma-associated antigen 3 (MAGE-A3) (228, 229). Both IGF2 and MAGE-A3 were selected from a panel of 12 genes and this two gene combination has a sensitivity of 81%, specificity of 91%, PPV of 87%, NPV of 88% and AUC of 0.944 in a prospective blinded validation study (228). The initial 12 gene expression targets were selected following screening using gene expression microarrays (228, 229). IGF2 represents glycoprotein receptors on the cell membrane IGF2 which promotes tumorigenesis via the PI3K-AKT pathway, and this is implicated in most bladder cancer (294). MAGE-A3 has been shown to be

expressed in 43% of bladder cancer and in various tumour types, but not in healthy tissue with the exception of testis and placenta (295, 296).

Table 5.7: Study characteristics and diagnostic accuracy of urinary transcriptomic biomarkers for the diagnosis of bladder cancer with sensitivity and specificity ≥80%.

Title	type of marker	Marker	test platform	Study design	Urine collection	Low Grade (%)	tumour arm	control arm	Sensitivity	Specificity	PPV	NPV	AUC
Ismail et al. 2016 (222)	Cytoplasmic calcium binding protein	S100A4	RT-qPCR	Case control	10ml void	16	120	30	90	92	89	93	0.978
De Martino et al. 2015 (223)	Zinc metalloenzyme	carbonic anhydrase IX	RT-qPCR	Case control	Not specified	56	83	72	81	96	96	81	0.883
Eissa et al. 2014 (224)	Cell-cycle regulating protein	hepatoma upregulated protein RNA	Gold nanoparticles RT-PCR	Case control	Voided urine	16	50	50	89	94			
Eissa et al. 2014 (225)	Cell-cycle regulating protein	hepatoma upregulated protein (HURP) + cytology	RT-qPCR	Case control	30-60 ml void	18	211	133	91	94	96	87	
Srivastava et al. 2014 (226)	Inhibitor of apoptosis protein	X-linked inhibitor of apoptosis protein (XIAP) + cytology	RT-qPCR	Case control	50 ml urine	25	117	74	98	93			
Schmidt et al. 2016 (227)	Inhibitor of apoptosis protein (surviving), Nuclear protein for cellular proliferation (Ki-67), Intermediate filament of urothelial cells (CK20)	CK20	RT-qPCR	Case control	50-200 ml urine	29	105	156	85	87			0.870
		Cytology + survivin							91	97			
		Cytology + CK20							97	90			
		ki67+ CK20							85	87			
Ribal et al. 2016 (228)	Growth factor (IGF2), melanoma-associated antigen (MAGE-A3), zinc finger transcription factor (KLF9), hormone (CRH), glutamate transporter (SLC1S6), POSTN-ligand to support cell adhesion and migration (POSTN), Catalytic subunit of telomerase enzyme (TERT), nuclear protein (AHNAK2), cel lular protein providing membrane scaffold (ANXAA10), protease (CTSE), protein for cellular structural integrity (KRT20); cellular protein that reverses serine/ threonine phosphorylation (PPP1R14D)	12 genes: IGF2, MAGEA3, KLF9, CRH, SLC1A6, POSTN, TERT, AHNAK2, ANXA10, CTSE, KRT20, PPP1R14D	RT-qPCR	Prospective consecutive observational	50-100 ml void	41	216	309	79	93	89	86	0.905
		10 genes: IGF2, MAGEA3, KLF9, CRH, SLC1A6, POSTN, EBF1, CFH, MCM10, MMP12							80	94	90	87	0.908
		5 genes: IGF2, MAGEA3, KLF9, CRH, SLC1A6							79	92	87	86	0.903
		2 genes: GF2, MAGEA3							81	91	87	88	0.918
Mengual et al 2014. (229)		12 genes: IGF2, MAGEA3, KLF9, CRH, SLC1A6, POSTN, TERT, AHNAK2, ANXA10, CTSE, KRT20, PPP1R14D	RT-qPCR	Prospective consecutive observational	50-100 ml void	Not specified	96	111	86	90	89	88	0.944
		10 genes: IGF2, MAGEA3, KLF9, CRH, SLC1A6, POSTN, EBF1, CFH, MCM10, MMP12							86	90	89	88	0.949
		5 genes: IGF2, MAGEA3, KLF9, CRH, SLC1A6							84	91	89	87	0.941
		2 genes: IGF2, MAGEA3							79	91	88	83	0.913
Salomo et al. 2017 (230)	Growth factor (IGF2), Intermediate filament of urothelial cells (CK20)	IGF2 + CK20	RT-qPCR	Case control	Voided urine	18	103	50	90	84	92	81	
		IGF2 + CK20 + cytology							93	82	91	85	
Eissa et al. 2015 (231)	Oncogenic long-non-coding RNA	long non-coding RNA urothelial carcinoma associated-1 (lncRNA-UCA1)	Nano assay RT-PCR	Case control	40 ml void	17	139	81	92	96	88	98	0.966
		lncRNA-UCA1 + cytology							97	96	95	98	

AHNAK2: AHNAK nucleoprotein 2; ANXA10: Annexin A10; CK20: cytokeratin 20; CRH: cortisol releasing hormone; CTSE: Cathepsin E; IGF2: insulin like growth factor; KLF9: Krueppel-like factor 9; KRT20: Keratin 20; MAGE-A3: Melanoma-associated antigen 3; MCM10: minichromosome maintenance complex component 10; MMP12: matrix metalloprotease 12; NPV: negative predictive value; POSTN: Periostin; PPV: positive predictive value; PPP1R14D: Protein phosphatase 1, regulatory (inhibitor) subunit 14D; SLC1A6: solute carrier family 1 member 6; TERT: Telomerase reverse transcriptase

Table 5.8: Study characteristics and diagnostic accuracy of urinary transcriptomic biomarkers for the diagnosis of bladder cancer with sensitivity and/ or specificity < 80%.

Title	Type of marker	Marker	Test platform	Study design	Urine collection	Low Grade (%)	Tumour arm	Control arm	Sensitivity	Specificity	PPV	NPV	AUC
Kim et al. 2016 (297)	DNA hydrolysis enzymes	Cell-free TopoIIA RNA	RT-qPCR	Case control	1 ml urine	21	83	115	74	68	64	79	0.741
Hattori et al. 2014 (298)	Cell surface protein	CD44 variant 6	RT-qPCR	Case control	5 ml void	5	21	25	86	72			0.777
De Martino et al. 2015 (299)	Serine/ threonine kinase	AURKA	RT-qPCR	Case control	50 ml void	57	122	66	84	65			
Gosalbez et al. 2014 (300)	Ligand for chemokine receptor CXCR4&7	SDF-1 alpha	RT-qPCR	Case control	Not specified	30	57	28	84	30			
Zhang et al. 2016 (301)	Enzyme involved in histone modification	EZH2 mRNA	RT-qPCR	Case control	0.5 ml urine	23	212	147	45	98			0.772
Kim et al. 2016 (302)	Ubiquitin-conjugating enzyme	UBE2C	RT-qPCR	Case control	1 ml urine	23	212	106	83	76			0.839
Milowich et al. 2015 (292)	Oncogenic long-non-coding RNA	Long non-coding RNA urothelial carcinoma associated-1 (lncRNA-UCA1)	RT-PCR	Case control	Not specified	33	30	49	84	65	86	61	
Srivastava et al. 2014 (303)	Oncogenic long-non-coding RNA	Long non-coding RNA urothelial carcinoma associated-1 (lncRNA-UCA1)	RT-qPCR	Case control	50 ml void	25	117	28	79	80	86	71	0.863
Gielchinsky et al. 2017 (304)	Long non-coding RNA regulating cell proliferation	Long non- coding RNA H19	RT-qPCR	Case control	50 ml void	86	21	27	96	67			0.933
Leotsakos et al. 2014 (305)	Inhibitor of apoptosis protein	Survivin	RT-qPCR	Case control	50 ml second void of day	8	105	39	21	95	92	31	
	Catalytic subunit of telomerase enzyme	TERT							11	100	100	29	
	Glycosylated phosphoprotein on B cells	CD20							33	97	97	35	
		Survivin + TERT positive							24	95	93	32	
		Survivin positive + CK20 positive							33	95	93	32	
		TERT + CK20 positive							36	97	97	36	
		Survivin + TERT+CK20 positive							36	95	95	36	

Kavalieris et al. 2015 (306)	IGFBP5- binding proteins for insulin growth factor; HOXA13- homeobox protein involved in transcription; MDK- Heparin binding growth factor, CDK1- cell cycle kinase, CXCR2- chemokine receptor	CX-bladder (G index): IGFBP5, HOXA13, Midkine, CDK1, CXCR2	Point of care test (RT-qPCR based)	Prospective visible haematuria patients	4.5 ml void				86			96	0.83
		Phenotype index (P-INDEX): age, gender, frequency of macrohematuria and smoking history											0.61
		G INDEX + P INDEX					72	515	95			98	0.86

IGF2: insulin like growth factor; MAGE-A3: Melanoma-associated antigen 3; KLF9: Krueppel-like factor 9; CRH: cortisol releasing hormone; SLC1A6: solute carrier family 1 member 6; POSTN: Periostin; TERT: Telomerase reverse transcriptase; AHNAK2: AHNAK nucleoprotein 2; ANXA10: Annexin A10; CTSE: Cathepsin E; KRT20: Keratin 20; PPP1R14D: Protein phosphatase 1, regulatory (inhibitor) subunit 14D; MCM10: minichromosome maintenance complex component 10; MMP12: matrix metalloprotease 12; CK20: cytokeratin 20

5.3.6 Combination of different 'omic' biomarkers

Ten studies used a combination of different 'omic' biomarkers with the aim to identify bladder cancer from exfoliated urinary bladder cells (Table 5.9 and Table 5.10). Six studies combined genomic with epigenetic biomarkers including one with microsatellite analysis (136, 232, 234, 307, 308). The other three studies used a transcriptomic and protein combination panel (235, 236, 309). One study utilised a protein (HYAL1), epigenetic (miR-210, miR-96) and transcriptomic (lncRNA-UCA1) combination. *TERT* and *FGFR3* mutation were used in most combination markers incorporating genomic biomarkers (136, 232, 234, 307).

In a retrospective analysis of case control study of 74 bladder cancer and 80 controls presenting with haematuria, a combination of *FGFR3*, *TERT* and *HRAS* mutation together with twist-related protein (*TWIST*), *OTX1* and *ONECUT2* methylation, reported sensitivity of 97% and specificity of 83% (232). The authors modelled a PPV of 39% and NPV of 99.6% assuming a 10% prevalence of bladder cancer (232). This six gene panel of epigenetic and genomic targets was subsequently validated in a prospective case control study with 97 bladder cancer and 103 controls presenting with haematuria had a sensitivity of 93% and AUC of 0.960 (233). This assay builds on a previously reported assay comprising of *FGFR3* mutation in combination with *OTX1, ONECUT2* and odd-skipped-related 1 (*OSR1*) methylation profile in a patient cohort of 95 cancers and 40 controls (136). This assay panel achieved a sensitivity of 79%, PPV of 92%, NPV of 76% and AUC of 0.864.

The other study by Dahmcke and colleagues was a prospective study which utilized a biomarker panel comprising of *FGFR3* and *TERT* promoter mutation with 6 methylated genes namely *ONECUT2*, Cyclin-A1 (*CCNA1*), *BCL2, EOMES*

and vimentin (*VIM*) (234). This 8-biomarker combination had sensitivity of 97%, specificity of 76.9%, NPV of 99% and AUC of 0.963 (234). Beukers and colleagues tested a three-panel biomarker comprising of *FGFR3* and *TERT* mutation with *OTX1* methylation and in pre-TURBT urine collection from 305 patients, achieved a sensitivity of 81-94% depending on tumour grade (307). However, in patients undergoing surveillance cystoscopy, the sensitivity and specificity of identifying tumour recurrence was much lower at 57-72% and 55-59% respectively (307).

A four-panel biomarker of *FGFR3* mutation with Heparan sulfate glucosamine 3-O-sulfotransferase 2 (*HS3ST2*), *SLIT2* or *SEPTIN9* methylation was tested in a cohort of patients for the identification of NMIBC recurrence with surveillance cystoscopy (310). Roperch and colleagues incorporated clinical features such as age and smoking which improved the diagnostic accuracy of the assay from a sensitivity of 67-89% to 98% depending on tumour grade with an AUC of 0.960 (310). However, when used in the surveillance setting, consistent with results from Beukers and colleagues, the sensitivity fell to 95% with an AUC of 0.820. Similarly, Zuiverloon and colleagues also observed that the diagnostic ability of urinary biomarkers to identify tumour recurrence during surveillance cystoscopy was poor (308).

The other three studies by Eissa et al. used combinations of protein and transcriptomics (235-237). Survivin involved in the EMT pathway was tested in combination with Matrix metalloproteinase (MMP) 2 & 9 and hyalurodinase. Survivin with MMP 2 & 9 had a sensitivity and specificity of 91% and 85% which increased to 96% and 85% when urinary cytology was incorporated (235). Sensitivity and specificity of survivin with hyalurodinase was 95% and 90%

respectively (236). The protein-epigenetic combination of HYAL1, lncRNA-UCA1, miR-210 with miR-96 had a sensitivity of 100%, specificity of 89% and AUC of 0.981 (237).

Table 5.9: Study characteristics and diagnostic accuracy of different combination 'omic' urinary biomarkers for the diagnosis of bladder cancer with sensitivity and specificity ≥80%.

Title	Type of marker	Marker	test platform	Study design	Urine collection	Low Grade (%)	tumour arm	control arm	Sensitivity	Specificity	PPV	NPV	AUC
Van Kessel et al. 2016 (232)	Epigenetic + genomic	Methylation: TWIST1, ONECUT2 and OTX1 Mutation analyses: FGFR3, TERT and HRAS	TWIST1- qMS-PCR OTX1 & ONECUT2- SNaPshot methylation assay, TERT, FGFR3, HRAS mutation- PCR	Case control	Not specified	20	74	80	97	83	23-39	100	0.930
Van Kessel et al. 2017 (233)	Epigenetic + genomic	Methylation: TWIST1, ONECUT2 and OTX1 Mutation analyses: FGFR3, TERT and HRAS	TWIST1- qMS-PCR OTX1 & ONECUT2- SNaPshot methylation assay, TERT, FGFR3, HRAS mutation- PCR	Prospective case control	Not specified	26	97	103	93	86		99	0.960
Dahmcke et al. 2016 (234)	Epigenetic + genomic	Methylation: SALL3, ONECUT2, CCNA1, BCL2, EOMES, VIM Mutation: TERT, FGFR3	SALL3, ONECUT2, CCNA1, BCL2, EOMES, VIM- methyl light TERT, FGFR3- Droplet digital PCR	Prospective observational consecutive blinded	Not specified	34	99	376	97	77	53	99	0.963
Eissa et al. 2013 (235)	Transcriptomic + protein	Survivin +MMP2&9	Survivin- RT-PCR MMP 2 & 9- zymography	Case control	30-60 ml void	G1/2: 76	46	20	91	85	88	89	
		Cytology + survivin							85	95	95	84	
		Cytology +MMP2&9							85	90	91	84	
		Cytology + survivin + MMP2&9							95	85	88	94	
Eissa et al. 2013 (236)	Protein + transcriptomic Protein: survivin Transcriptomic: Hyaluronidase	Hyaluronidase	Survivin- ELISA Hyaluronidase- RT-PCR	Case control	30-60 ml void	G1/2: 79	60	40	87	98	83	98	
		Survivin + cytology							83	83	77	88	
		Hyaluronidase + cytology							90	98	87	98	
		Survivin + hyaluronidase							93	90	90	93	
		Survivin + hyaluronidase + cytology							95	90	92	93	
Eissa et al. 2015 (237)	Protein + epigenetic + transcriptomic Protein: HYAL1 Transcriptomic: lncRNA-UCA1 Epigenetic: miR-210, miR-96	HYAL1	HYAL1- zymography miR-210 + miR96- RT-qPCR lncRNA-UCA1- RT-qPCR	Case control	40-60 ml void	17	94	116	89	91	89	91	0.948
		lncRNA-UCA1							92	97	96	93	0.975
		HYAL1 + miR-210+ miR96+ LucRNA-UCA1+ cytology							100	90	88.7	100	0.981

BCL2: B-cell lymphoma 2; CCNA1: Cyclin A1; EOMES: Eomesodermin; FGFR3: fibroblast growth factor receptor 3; HYAL1: Hyaluronoglucosaminidase 1; lncRNA-UCA1: long non-coding RNA-urothelial cancer associated 1; MMP2: matrix metalloproteinase-2; MMP9: matrix metalloproteinase-9; NPV: negative predictive value; ONECUT 2: One Cut Homeobox 2; OTX1: orthodenticle homeobox 1; PPV: positive predictive value; SALL3: spalt-like transcription factor 3; TERT: Telomerase reverse transcriptase; TWIST1: Twist Family BHLH Transcription Factor 1; VIM: Vimentin

Table 5.10: Study characteristics and diagnostic accuracy of different combination 'omic' urinary biomarkers for the diagnosis of bladder cancer with sensitivity and/ or specificity <80%.

Title	Type of marker	Marker	Test platform	Study design	Urine collection	Low Grade (%)	Cancer arm	Control arm	Sensitivity	Specificity	PPV	NPV	AUC
Kandimalla et al. 2013 (136)	Methylation	OTX1	a multiplex bisulphite-SNaPshot assay	Case control	25-100 ml void	28	140	70	65		90	65	0.805
		MEIS1							46		87	54	0.749
		ONECUT2							52		88	57	0.737
		SIM2							49		88	56	0.753
		FOXA1							38		84	50	0.659
		ZNF503							52		88	57	0.784
		HOXA9							62		90	63	0.829
		OSR1							44		86	53	0.705
	Mutation	FGFR3	PCR						52		100	58	0.762
	Methylation	Methylation: OTX1, ONECUT2, OSR1				35	95	40	74		91	72	0.864
	Methylation + mutation	Methylation: OTX1, ONECUT2, OSR1 + mutation: FGFR3							79		92	76	0.886
	Methylation	Methylation: OTX1+ONECUT2+OSR1+ cytology							77		89	79	0.89
	Methylation + mutation	Methylation: OTX1+ONECUT2+OSR1+ cytology + mutation: FGFR3							82		89	83	0.904
Beukers et al. 2017 (307)	Mutation (FGFR3, TERT), methylation (OTX1)	Mutation: FGFR3, TERT + methylation: OTX1	PCR (mutation), Bisulphite Specific-SnaPshot (methylation)	Primary tumour	Voided collection	47	305		81-94				
		Mutation: FGFR3		Prospective cohort			382 cancer recurrence of 2191 surveillance urine samples		12- 27	91- 95			
		Mutation: TERT							45- 61	63- 70			
		Methylation: OTX1							18- 38	78- 81			
		Mutation: FGFR3, TERT + Methylation: OTX1							57- 72	55- 58			
Eissa et al. 2013 (309)	Transcriptomic (hTERT) + Protein (scatter field)	Scatter factor	RT-PCR (hTERT) + ELISA (scatter field)	Case control	Voided collection	40	60	45	95	78			0.857
		TERT+ scatter factor							98	78			
Zuiverloon et al. 2013 (308)	Methylation (APC, TERT, EDNRB) + genomic (FGFR3 mutation, microsatellite analysis)	Mutation: FGFR3	PCR (FGFR3, microsatellite analysis), MRC kit-001-using hybridization of probes to CpG site (methylation)	Prospective cohort	10- 50 ml void	25	136 cancer patients with a median of 3 year follow up with 716 urine samples (552 recurrence)		49				
		Mutation: FGFR3 + microsatellite analysis							79				
		Mutation: FGFR3 + methylation assay (APC, TERT, EDNRB)							75				

CCNA1: Cyclin A1; EDNRB: Endothelin receptor type B; EOMES: Eomesodermin; FGFR3: fibroblast growth factor receptor 3; FOXA1: Forkhead box protein A1; HOXA9: Homeobox A9; HYAL1: Hyaluronoglucosaminidase 1; lncRNA-UCA1: long non-coding RNA-urothelial cancer associated 1; MMP2: matrix metalloproteinase-2; MMP9: matrix metalloproteinase-9; NPV: negative predictive value; ONECUT 2: One Cut Homeobox 2; OTX1: orthodenticle homeobox 1; OSR1: odd-skipped-related 1; PPV: positive predictive value; SALL3: spalt-like transcription factor 3; SIM2: Single-minded homolog 2; TERT: Telomerase reverse transcriptase; TWIST1: Twist Family BHLH Transcription Factor 1; VIM: Vimentin; ZNF503: Zinc finger protein 503

5.4 Discussion

This systematic review highlights that single target assays have limited value regardless of 'omic' class. Performance is uniformly below that of multi-target biomarker panels. Only 4 single target urinary biomarkers achieved a sensitivity and specificity of ≥90% (Table 5.11). Across all studies none had a pre-planned statistical power calculation performed with only four non-case controlled prospective observational studies (220, 228, 229, 234). A total of six studies using two different biomarker panels performed independent validation studies. The first, a 10 protein based multiplex assay (IL8 + SERPINA1 + ANG + VEGF-A + CA9 + MMP 9 & 10 + APOE + PAI-1 + SDC1) and the second, a two panel gene expression assay (IGF2, MAGEA3)(201-204, 228, 229). Both assays reported a sensitivity and specificity of <90% and AUC of <0.950. One panel comprising of 6 DNA methylation *(SALL3* + *ONECUT2* + *CCNA1* + *BCL2* + *EOMES* + *VIM*) and two mutation (*TERT* & *FGFR3*) were field-tested in a prospective blinded cohort of haematuria patients. The authors reported a sensitivity, specificity and AUC of 97%, 77% and 0.963 respectively but the panel was not validated in a prospective independent patient cohort (234). A significant number of studies on urinary biomarkers have poor diagnostic ability and require validation in a prospective clinical setting.

Table 5.11: Urinary biomarkers stratified according to 'omic' class and single vs multiple target biomarker with sensitivity and specificity of ≥80%.

Promising single biomarkers	
Protein	• Orosomucoid 1 (ORM1)* • Survivin • APE1/Ref-1 • Soluble FAS • HtrA1* • Apo-A1 • Calprotectin • Nuclear matrix protein 52 • Ubiqutin 2 • Hyaluronidase • Hyaluronic acid • DJ-1/PARK7 • Interlukin-8 • Uroporphyrin I • Coproporphyrin • AIB1
Genomic	• *TERT*
Epigenetic	• POU Class 4 Homeobox 2* • Transcription factor 21
Transcriptomic	• S100A4 • Carbonic anhydrase IX • Hepatoma upregulated protein RNA • Cytokeratin 20 • Long non-coding RNA urothelial carcinoma associated-1*
Promising biomarker combination	
Protein	• Amplified in breast cancer 1 + eukaryotic initiation factor 2 + Nuclear matrix protein 22 • Apolipoprotein A1 + cytology • Cytology+ midkine + gamma synuclein* • Hyaluronic acid + hyaluronidase • Coronin-1A + Apolipoprotein A4 + Semenogelin-2 + synuclein-g + PARK7/ DJ-1* • Interleukin 8+ Matrix metallopeptidase 9 + Vascular endothelial growth factor A • Interleukin 8 + SERPINA1 + ANG + Vascular endothelial growth factor A + CA9 + Matrix metallopeptidase 9 & 10 + Apolipoprotein E + Plasminogen activator inhibitor-1+ Syndecan ǂ • Spectral range- 1500-1340, 1100-900, 900-800 • Metabolic signature- succinate, pyruvate, oxoglutarate, carnitine, phosphoenolpyruvate, trimethyllysine, melatonin, isavalsrylcarnitine, glytarylcarnitine, octenoylcarnitine, decanoylcarnitine, acetyl-coA*

	• Metabolic signature- GlyCysAlaLys, Inosinic acid, Trehalose, Nicotinuric acid, Asp Asp Gly Trp, Ureidosuccinic acid • Principal component analysis*
Epigenetic	• mRNA-99a +mRNA-125b • MiR-96+ cytology • miR-187 + miR-18a + miR-25 + miR-142-3p + miR-140-5p + miR-204 • 10 and 25 panel miR • Cell free: miR-7-5p + miR-22-3p + miR-29a-3p + miR-126-5p + miR-200a-3p + miR-375 + miR-423-5p • Methylation: SOX1 + Interleukin 1 Receptor Associated Kinase 3 + Line 1 MET • Methylation: POU Class 4 Homeobox 2 + Protocadherin-17* • Methylation: 150 CpG sites*
Transcriptomic	• Hepatoma upregulated protein + cytology* • X-linked inhibitor of apoptosis protein + cytology* • Cytokeratin 20 + cytology* • Survivin + cytology* • Ki67 + Cytokeratin 20 • Insulin like growth factor 2, Melanoma-associated antigen 3 ǂ • Cytokeratin 20 + Insulin like growth factor 2 • Long non-coding RNA urothelial carcinoma associated-1 + cytology*
Multi 'omic' biomolecule	• Methylation: Twist Family BHLH Transcription Factor 1, One Cut Homeobox 2 + orthodenticle homeobox 1. Mutation: Fibroblast growth factor receptor 3, Telomerase reverse transcriptase and HRAS ǂ • Methylation: Spalt-like transcription factor 3 + One Cut Homeobox 2 + Cyclin A1 + B-cell lymphoma 2 + Eomesodermin + Vimentin. Mutation: Telomerase reverse transcriptase + Fibroblast growth factor receptor 3 • Matrix metalloproteinase 2 & 9 (protein) + survivin (mRNA) + cytology* • Survivin (protein) + hyaluronidase (mRNA) + cytology* • HYAL1 (protein) + miR-210 + miR96+ long non-coding RNA-urothelial cancer associated 1 (mRNA) + cytology*

*≥90% sensitivity and specificity

ǂ independent cohort validation studies

There is considerable interest in the use of urinary biomarkers to diagnose bladder cancer. This applies to both patients evaluated for haematuria, as well as NMIBC patients undergoing surveillance cystoscopy. The requirement for cystoscopy to diagnose bladder cancer represents a significant cost to health care services (311). Further, cystoscopy requires a hospital visit and is an invasive procedure with a risk of UTI (20). A highly sensitive and specific non-invasive urinary assay has the potential to revolutionise both the haematuria and NMIBC surveillance pathways.

I report that the diagnostic accuracy of urinary biomarkers varies considerably. Single target biomarkers had a sensitivity of between 2-94%, specificity of 46-100%, PPV of 47-100% and NPV of 21-94%. Multi-target biomarkers achieved sensitivities of 24-100%, specificities of 48-100%, PPV of 42-95% and NPV of 32-100%. Such variations in diagnostic accuracy can be explained by combination of cancer specific factors and assay factors. The diagnostic ability of urinary biomarkers was considerably better in identifying high grade tumours as well as CIS. This is similar to urine cytology which has an overall sensitivity of 34% and specificity of 99% but the sensitivity increases to 63% in CIS and high grade tumours (159). The increased exfoliation of tumour cells in these cancers may in fact reflect why novel urinary biomarkers also detect high grade disease with a higher sensitivity and specificity. In fact, ≥pT2 bladder cancer is often associated with a high mutational burden and hypermethylation (312).

Beside cancer specific variables, reproducibility of biomarkers to allow clinical use remains an issue. While efforts are made by the implementation of Good Laboratory Practice to uphold the quality of management controls to ensure consistency and reliability of results, there are other sources of variation even

when the same biomarker is interrogated. The existing variation in evaluating the same target protein, epigenetic change or gene expression makes it difficult to compare studies due to the lack of standardization of methodology (313). NGS performed in 5 different centres of the International Cancer Genome Consortium (IGGC) suggests that differences in variant calling and complete sequencing pipelines can result in differences in the identification of ≥75% of mutations (314). Further, variation in genetic differences such as mutation, post transcription modifications, gene expression and epigenetic changes are complex and is difficult to elucidate. Additionally, the threshold used to define a positive result may differ between studies making comparison difficult.

A significant number of biomarkers reported did not have external validation in prospective field testing. For reasons described above, the diagnostic accuracy of initial reports is often not reproducible. When validation was performed, it was typically performed using selected patient cohort which is not representative of 'real world practice' of haematuria patients or NMIBC patients undergoing surveillance cystoscopy. The majority of studies were based on retrospective patient cohorts comprising of selected bladder cancer and control patient groups. Hence, accurate PPV and NPV cannot be determined accurately as PPV and NPV are dependent on the prevalence of disease in the patient cohort.

This study shows that the use of multi-target biomarkers is increasing and these biomarker panels have in general a higher accuracy (Table 5.11). Traditionally, the number of biomarkers incorporated in an assay has been limited by DNA. Generally, female patients have a higher DNA yield compared to male patients (315). In addition, DNA extraction kit used and sampling time can also affect the DNA quality and quantity (315). Particularly in methylation based assays which

requires DNA bisulphite conversion and can results in the loss of up to 70-90% DNA (316). Fluorometer quantification of urinary DNA suggest that between 2 to 440 ng/ ml of DNA can be retrieved from urinary cell pellet (315). In the studies reviewed, the limit on biomarker targets interrogated for protein, genomic, epigenetic, transcriptomic and combination biomarkers are 10, 5, 150, 12 and 8 respectively. The utility of NGS has allowed the development of highly multiplex assays, for genomic, epigenomic or transcriptomic biomarkers. The first to utilise this technology used multiplex biomarker panel of 150 loci (140).

The use of multi-target biomarkers is supported by seminal studies suggesting that there is significant intra-tumour heterogeneity within the same primary tumour (317). Hence, the diagnostic accuracy of biomarkers can be improved by a multitarget approach, it is unlikely that a single biomarker will be able to achieve a high diagnostic accuracy which meets the expectations of patients (318). While it is established that common mutations such as *FGFR3* and *TERT* are common in NMIBC, even in combination, a *FGFR3,TERT* mutation assays will miss >20% of bladder cancers (273).

Currently, large multi-panel biomarkers are identified using next generation sequencing or arrays followed by a validation cohort of patients. However, incorporating more biomarkers into a panel may not improve diagnostic accuracy (209, 228, 229). The traditional methods of defining a positive test using a score and benchmarking it against an arbitrary threshold when evaluating multiple biomarkers is not ideal. Additionally, the choice of biomarkers to be incorporated is key. Using multiple highly sensitive and specific biomarkers with significant overlap may not improve diagnostic performance. Hence, modern approaches incorporating complex bioinformatics and machine learning approaches using big

data analysis represents a step change approach (319). Mathematical models such as random forest classifier or network models allow the aggregation of highly sensitive and specific biomarkers, resulting in a more robust test. In addition, considering KEGG pathways to determine truncal biological pathways implicated in bladder cancer carcinogenesis may allow for better biomarker selection which reflects functional biology (320). Further, aggregating different 'omic' biomarkers such as simultaneous analysis of DNA methylation, mutation, gene expression and copy number alterations has been hypothesized to improve biomarker accuracy (321). This approach has been utilised by two groups combining genomic with DNA methylation targets to achieve an AUC of 0.960 (233, 234). Several studies also incorporated urinary cytology in addition to other biomarkers which resulted in improved biomarker performance (199, 226, 230, 231). Combining standard radiological images with genetic analysis has also proven to be an effective strategy in biomarker development (322).

The acceptable threshold of a urinary biomarker is dependent on its use as a companion test or a definitive test to replace cystoscopy. The sensitivity of a urinary assay used to replace cystoscopy in the haematuria setting should be high given the devastating consequences in missing a bladder cancer particularly high-risk disease. Historic patient surveys suggest that patients would only consider a urinary test with a diagnostic sensitivity of ≥95% (318).

5.5 Limitations

The current study is not without limitations. In the systematic review, I reviewed the published literature since 2013 hence reported biomarkers with a high diagnostic accuracy published before 2013 will not be captured. However, given that no urinary biomarker has the diagnostic ability to replace cystoscopy, I would expect that validation studies of promising biomarkers would continue to be reported. As with most studies, positive results are often reported, and negative results remain unpublished hence there may be many more biomarkers investigated but they are likely to be of limited value.

5.6 Conclusions

The field of urinary biomarkers for the detection of bladder cancer is rapidly developing. However, no biomarkers reported to date can replace cystoscopy. The lack of field testing, validation studies, and tumour heterogeneity represents challenge to biomarker development and validation. However, NGS with the use of complex machine learning and mathematical modelling may represent a promising approach for biomarker discovery, and promising biomarkers should be field tested to validate them.

CHAPTER 6 : DIAGNOSTIC ABILITY OF THE UROMARK

6.1 Introduction

Changes in DNA methylation play a central role in malignant transformation, leading to the silencing of tumour-suppressor genes and overexpression of oncogenes (323). Despite its plasticity, DNA methylation is oncogenically stable, a property which can be exploited to identify potential diagnostic biomarkers. The utility of DNA methylation alterations as urinary biomarkers for the detection of bladder cancer, in both primary and recurrence settings, is a highly active area of research (324). Although several urinary epigenetic biomarker studies have shown promising, they have not reached the required sensitivity or specificity to replace cystoscopy. This is particularly true for recurrent NMIBC, where the sensitivity for the detection of recurrent bladder cancer has been in the range of 66-68%, even with the addition of somatic mutation, such as *FGFR3,* or urinary cytology (308).

A systematic review performed in Chapter 5 reported that small panel assays have shown limited success. However, the number of loci which can be included within a panel using conventional technologies to detect methylation and / or somatic mutation has been the limiting factor for assay development. This is due to limited amount of DNA yield from voided urine and the technical issues around running multiple assays form a single sample. The development of novel technologies, such as micro-droplet PCR combined with next-generation bisulfite sequencing, has overcome some of these issues, allowing the interrogation of a large panel of epigenetic (or somatic) biomarkers from a single sample.

The Kelly-Feber laboratory have developed the UroMark assay, which uses high-throughput targeted bisulphite sequencing to detect bladder cancer specific alterations in DNA from urine sediment cells. The UroMark assay uses a micro-

droplet PCR platform (Thunderstorm, RainDance Technologies), which allows the simultaneous amplification of a panel of 150 loci (325). The UroMark loci were identified following genome wide methylation analysis of 81 primary bladder cancer (tumour content >80%) and 30 aged matched normal urothelium samples using the Infinium HumanMethylation 450k array (Illumina, San Diego, USA), and has been previously reported (325). For loci to be included in the UroMark assay, they had to be unmethylated in normal, blood (sorted cell populations which includes immune cells and whole bloods) and non-cancer urines, and highly methylated in cancers. Utilising these cut offs, a well-defined bladder cancer specific panel of molecular markers was developed for the detection of bladder cancer using urinary cellular content.

In this chapter, I report an interim analysis of the prospective validation study to test the diagnostic performance of the UroMark in the haematuria setting. I performed some of the DNA extraction from the urine samples. Bisulfite conversion of DNA, RainDance microdroplet PCR, targeted methylation sequencing and bioinformatics analysis were performed by Dr Liqin Dong (post-doctorate researcher, UCL Cancer Institute) and Dr Andrew Feber (senior lecturer, UCL Cancer Institute). The final output of each urine sample was provided to me as positive or negative.

6.2 Methods

6.2.1 Patient selection

Between April 2016 and May 2017, 80 patients were prospectively recruited from 32 hospitals in England. Patient inclusion criteria has been previously reported in Chapter 3.2.1. This represents an interim analysis of the performance of the UroMark assay. This interim analysis was not pre-planned and was permitted with the permission of the DETECT I chief investigator (John D Kelly) and the Surgical & Interventional Trials Unit (SITU) for the purpose of this thesis submission. An embargo of this thesis will be applied for until the results of the primary analysis of DETECT I is published.

6.2.2 Interventions

Clinical evaluation of patients has been previously described in Chapter 3.2.2.

6.2.4 Logging of urine samples

Details of urine collection has been previously described in Chapter 2.2.1.3. Recording and linking of urine samples with clinical data was performed using a custom designed database by Dr Simon Rodney (Urology research fellow, UCL Cancer Institute) (www.urologytrials.com). Each urine sample received was allocated a unique universal patient identifier (UPI) and a universal sample identifier (USI). A patient specific UPI was generated for each patient, but each sample collected from the same patient was allocated a unique USI. The

DETECT number, UPI and USI is recorded on each urine sample to ensure multiple identifiers.

6.2.6 Processing of urine samples

Urine was centrifuged to obtain a urine cell pellet and DNA extraction and quantification were predominantly performed by Miss Sheida Razee (laboratory assistant, UCL Cancer Institute). Bisulfite conversion of DNA, RainDance microdroplet PCR, targeted methylation sequencing and bioinformatics analysis were performed by Dr Liqin Dong (post-doctorate researcher, UCL Cancer Institute) and Dr Andrew Feber (senior lecturer, UCL Cancer Institute).

6.2.6.1 Centrifuge of urine to obtain urine cell pellet

Urine cellular content was pelleted by centrifugation at 1500 g for 10 minutes and the supernatant removed. The cell pellet was then washed with 500 µl of phosphate buffered saline (PBS) and repelleted again by centrifugation at 1500g for a further 2 minutes. The cell pellet was then stored at -20 ºC prior to DNA extraction.

6.2.6.2 DNA extraction & quantification

DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Cell pellet was washed with PBS and repellected. The cell pellet was digested using 20 µl of Proteinase K and mix by vortex. The samples were then

incubated overnight in a rotating incubator at 37ºC. 200 µl of Buffer AL and 200 µl of ethanol (96-100%) were subsequently added to lysed sample and vortexed. The mixture was transferred into a DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at ≥ 6000 g (8000 rpm) for 1 minute. The spin column was then transferred to a new 2 ml collection tube and 500 µl of Buffer AW1 was added and centrifuged for 1 minute at ≥ 6000 g (8000 rpm). The mini spin column was then placed in the final collection tube and 500 µl of Buffer AW2 was added and centrifuged for 3 minutes at ≥20,000 g (14,000 rpm) to dry the DNeasy membrane. The spin column was then placed in a clean 2 ml microcentrifuge tube and 100 µl of Buffer AE was pipetted directly onto the DNeasy membrane and incubated at room temperature for 1 minute and centrifuged for 1 minute at ≥6000 g (8000 rpm) to elute. DNA was then stored at -20ºC until used.

DNA was quantified by spectrophotometry (Nanodrop 1000, Thermo Fisher Scientific, Waltham, MA, USA) and fluorimetry (Qbit dsDNA HS Assay Kit, Invitrogen, Carlsbad, CA, USA). DNA integrity was assessed using a Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA)

6.2.6.3 Bisulfite conversion of DNA and epigenetic analysis

Bisulfite conversion of DNA was performed using EZ DNA Methylation Kit (Zymo Research, Irvine, CA, USA) according to manufacturer's instructions. Following bisulfite conversion, microdroplet PCR amplification was performed using the ThunderStorm system (RainDance Technologies). Targeted 200 bp single end methylation DNA sequencing using the Illumina Hi-seq or Mi-seq (Illumina Inc,

San Diego, USA) was performed. Approximately 1 million reads/ sample, with a minimum of death coverage of 1000X was performed. Sequence data quality was assessed using FastQC, and poor quality bases and reads removed using Trimgalore (178). Sequence alignment and methylation level was carried out using Bismark (326). Resulting methylation scores was then fed into the UroMark analysis pipeline to determine the likely presence of cancer using a random forest classifier approach.

6.2.7 Statistical analysis

Continuous data were reported as descriptive statistics using mean, median, interquartile range and 95% confidence interval. Categorical variables were compared using Chi-square test and continuous variables were analysed using t-test. Normal distribution was assumed. Sensitivity, specificity, PPV and NPV were calculated for correct identification for bladder cancer or upper tract TCC. SPSS v22 (IBM Corp, Armonk, New York, USA) was used to perform all statistical analysis. Statistical significance was set at p value <0.05

6.3 Results

6.3.1 Patient demographics

At interim analysis, a total of 80 patients investigated for haematuria from the DETECT I study were assayed (Cohort 1). Four samples (4.8%) did not have sufficient DNA yield for the assay. There were 24 (30.0%) bladder cancers and 1 (1.3%) RCC and 0 UTUC in this cohort. Other diagnosis includes 1 patient with prostate cancer. Patient demographics for this cohort is shown in Table 6.1.

Table 6.1: Patient demographics of patients investigated for haematuria (Cohort 1).

Variable	**N=80**
Age, median (IQR)	72.7 (62.0, 80.4)
Gender, n (%):	
Male	52 (65.0)
Female	28 (35.0)
Type of haematuria, n (%):	
Visible	57 (71.3)
Non-visible	23 (28.7)
Smoking history, n (%):	
Non-smoker	28 (35.0)
Ex-smoker	36 (45.0)
Current smoker	12 (15.0)
Not known	4 (5.0)
Bladder cancer incidence, n (%)	24 (30.0)
Upper tract urothelial cancer, n (%)	0 (0)

Bladder cancer characteristics are shown in Table 6.2. Histologically confirmed pTa cancers were predominant (n=12) with 4 (16.7%) low risk cancers. Urothelial cell carcinoma accounted for 87.5% of cancers.

Table 6.2: Bladder cancer histopathological characteristics in Cohort 1.

Variables	N=24
Tumour grade, n (%):	
G1	4 (16.7)
G2	9 (37.5)
G3	11 (45.8)
Tumour stage, n (%):	
pTa	12 (50.0)
pT1	6 (25.0)
≥pT2	6 (2.5)
Tumour risk classification, n (%):	
Low	4 (16.7)
Intermediate	8 (33.3)
High	12 (50.0)
Bladder cancer type:	
TCC	21 (87.5)
SSC	3 (12.5)

For the sensitivity analysis, the 24 bladder cancers in Cohort 1 were enriched with a further 22 cases with a visual diagnosis of bladder cancer from DETECT II study (Cohort 2). 18 of the 22 cases had histologically confirmed bladder cancer with four patients having a non-cancer diagnosis, including one patient with a benign papilloma.

Table 6.3: Enriched bladder cancer histopathological characteristics

Variables	N=42
Tumour grade, n (%):	
G1	4 (9.5)
G2	16 (38.1)
G3	22 (52.4)
Tumour stage, n (%):	
pTa	23 (54.8)
pT1	10 (23.8)
≥pT2	9 (21.4)
Tumour risk classification, n (%):	
Low	4 (9.5)
Intermediate	15 (35.7)
High	23 (54.8)
Bladder cancer type:	
TCC	36 (85.7)
SSC	5 (11.9)
Adenocarcinoma	1 (2.3)

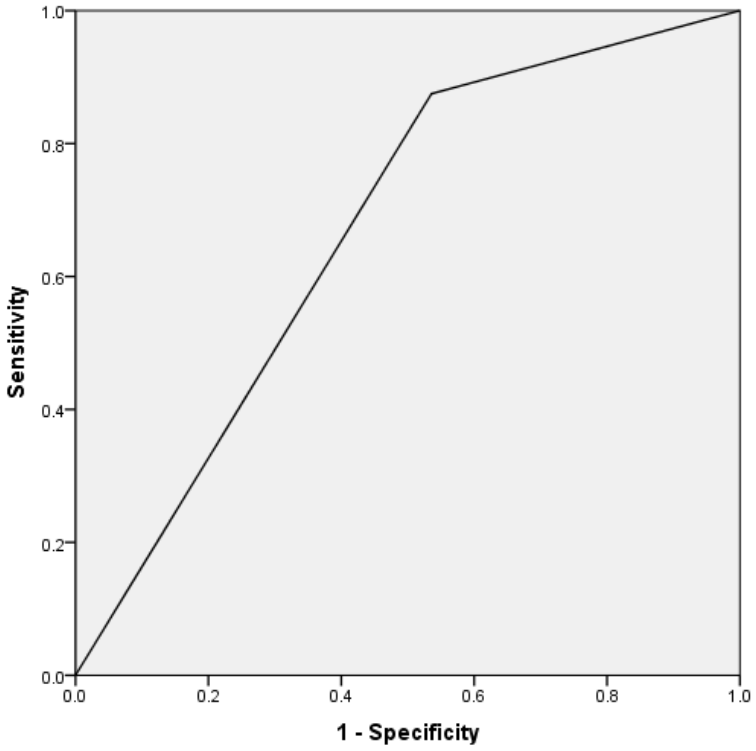
6.3.2 Diagnostic accuracy of the UroMark

The assay performance for the 80 patients investigated for haematuria were evaluated as part of an interim analysis of 10% of patients required for the study. The UroMark achieved a sensitivity of 87.5%, specificity of 46.4%, PPV of 40.4% and NPV of 92.9%. This corresponds to an AUC of 0.670.

Table 6.4: Two by two table of haematuria patients with or without bladder cancer against UroMark positive and negative

	Bladder cancer on histology	**No histologically confirmed bladder cancer**	**Total**
UroMark positive	21	30	52
UroMark negative	3	26	28
Total	24	56	80

Figure 6.1: Receiver operator characteristics curve of for UroMark for the detection of bladder cancer. AUC: 0.670.



The UroMark assay identified all patients with ≥pT1 stage and high-risk cancers. All three patients with a false negative result had solitary G2pTa and intermediate risk cancer. One of the three cancers was identified following CTU imaging. The remaining two cancers were <1 cm papillary lesions.

Of the 30 cases with false positive results, none had a diagnosis of upper tract cancers. Non-smokers, ex-smokers and current smokers comprise of 13 (43.3%), 11 (36.7%) and 5 (16.7%) patients respectively. Median age was 68.6 (IQR: 57.1-75.2) which was not significantly different from the entire patient cohort. One patient had a diagnosis of pT3 high risk prostate cancer. There was no benign bladder lesion identified in this cohort.

6.3.3 Sensitivity analysis of UroMark

The interim sensitivity analysis was performed for Cohort 2. Bladder cancer clinical- pathological characteristics for the 42 cancers are shown in Table 6.3. The UroMark identified 39 of the 42 cancers with a sensitivity of 92.9%.

6.4 Discussion

In this chapter, I report the interim analysis of the UroMark assay in patients investigated following a presentation of haematuria. The UroMark represents a non-invasive urine-based methylation assay for the detection of bladder cancer. For the first time, the utility of microdroplet PCR and NGS allows the interrogation of a high number of targets to achieve a urine biomarker with a maximum sensitivity.

The UroMark has a sensitivity of 100% for the detection of high risk bladder cancer. All bladder cancers ≥pT1 were identified by this assay. Overall, the UroMark achieved a sensitivity of 87.5%, specificity of 46.4%, PPV of 40.4% and NPV of 92.9% in the interim analysis of 80 patients presenting with haematuria. An enriched patient cohort of 42 bladder cancer patients achieved a sensitivity of 92.9%. This represents a much higher sensitivity compared to sensitivity achieved from current FDA approved biomarkers (47-85%), although the specificity of the UroMark is lower (53-95%) (170).

The utility of combining both DNA methylation and mutation panels is not new. Dahmcke et al. reported results of a 8-biomarker panel comprising of *FGFR3* and *TERT* mutation with 6 methylated genes namely *ONECUT2*, Cyclin-A1 (*CCNA1*), *BCL2, EOMES* and vimentin (*VIM*) in a prospective study (234). This biomarker panel achieved a sensitivity of 97%, specificity of 76.9%, NPV of 99% and AUC of 0.963 (234). Van Kessel et al. reported a retrospective analysis of case control study of 74 bladder cancer and 80 controls presenting with haematuria, investigating a combination of *FGFR3*, *TERT* and *HRAS* mutation in combination with twist-related protein (*TWIST*), *OTX1* and *ONECUT2* methylation, and reported a sensitivity of 97% and specificity of 83% (232). These studies were

much smaller patient cohorts compared to the DETECT I study, and it would be interesting to compare final results of the DETECT I study when assay of urine samples is completed.

DNA methylation is highly cell and tissue specific, and changes in DNA methylation have been shown to be some of the earliest events in carcinogenesis (327). Furthermore, DNA hypermethylation has been shown to be highly consistent across cancers, suggesting that DNA methylation would be an ideal biomarker for cancer detection (328).

The UroMark represents a novel approach to biomarker discovery. It encompasses a large number of both methylation and mutation targets. As discussed in Chapter 5, biomarkers with a higher number of targets frequently outperform oligo-markers. Previous limitation of using high number of multiplex targets was due to amount of substrate DNA available in voided urine. The utility of the RainDance microdroplet PCR platform allows for PCR amplification of up to several hundred targets. This in combination with next generation bisulfite sequencing allows for 150 methylated loci, which were selected following genome-wide DNA methylation screening of 260 bladder cancer cases. Using such a high number of targets is necessary to overcome issues with tumour heterogeneity where cancers may harbour non-truncal methylation and mutations (329).

However, the UroMark did miss three intermediate risk bladder cancer cases, two of which were bladder cancers <1 cm in diameter. This was not related to patient age or smoking history. Analysis of DNA input suggest that DNA integrity was adequate and not related to assay efficiency due to steps taken to maintain quality assurance and the appropriate controls. It is plausible that these cancers

did not harbor somatic mutations or DNA methylation signature interrogated by the UroMark assay. Formalin-Fixed Paraffin-Embedded (FFPE) tumour blocks have been requested from the recruiting hospital with the intention of extract tumour DNA for UroMark assay analysis to determine if the tumour tissue itself has DNA mutations and DNA methylation loci interrogated by the UroMark.

The ability for such rapid accruement of 2,679 urine samples over 15 months is testament that the NHS is well equipped and can deliver in biomarker studies. However, equally important is the urine collection kit which has been developed in- house in our laboratory. The urine collection kit allow patients to collect urine at the convenience of their home and utilises the Royal Mail to deliver it to the receiving laboratory. My preliminary results show that urine collection with the preservative agent requires only 75 ml of urine to provide adequate DNA yield for the UroMark multiplex assay. Further, DNA integrity remains stable for up to 8 days following voiding (personal communication Dr Patricia deWinter).

6.5 Limitations

Several limitations exist. Unlike point of care commercially available multiplex assays such as the Xpert Bladder Cancer Monitor (Cepheid, Sunnyvale, USA) which produces an output within 90 minutes, the UroMark pipeline requires a significant number of steps (330). The current pipeline involves centrifuging urine to obtain the urine cell pellet, from which DNA is extracted. Extracted DNA is then bisulfite converted followed by amplification. The amplified libraries are sequenced and processed using a bioinformatics pipeline. This complex pipeline requires a minimum of 1 week from receipt of urine to assay result hence a delay in reporting. However, this would still be faster than the 2-week wait cancer referral target which is the standard of care in the NHS. The complexity of the UroMark assay suggest that there would also be associated cost in the delivery of the potential service. Besides staff cost for a clinical scientist and a laboratory technician and associated sequencing, the current cost price of the UroMark is approximately £150 per sample. This will no doubt decrease with economies of scale.

6.6 Conclusion

The results of the interim analysis suggest that the sensitivity of the UroMark is within expectations. The utility of NGS coupled with machine learning represents a novel approach to diagnostic biomarker development. A non-invasive urine-based biomarker for the detection of bladder cancer will revolutionise the investigation and promote early detection of bladder cancer.

CHAPTER 7 : THE DEVELOPMENT AND EXTERNAL VALIDATION OF THE HAEMATURIA CANCER RISK SCORE TO DETERMINE RISK OF BLADDER CANCER

7.1 Introduction

The decision to guide who should have investigations following a presentation of haematuria varies between guidelines (26). Consistent across guidelines is the use of age specific thresholds to guide referral for investigation of VH and NVH, as increasing age is an established risk factor for bladder cancer. Adopting arbitrary thresholds will invariably result in an increased likelihood of missed cancers, along with the over investigation of cases unlikely to harbour malignancy. In Chapter 3, I reported that 3.5% of patients presenting with VH and 1.0% of patients with NVH where diagnosed with malignancy despite not meeting the age threshold set out in NICE guidance, suggesting that age thresholds and type of haematuria alone is not sufficient to determine who should be investigated for haematuria (143).

Predictive and prognostic tools using statistical models have been developed in the form of nomograms enabling individual patient-specific risk estimation (331). Nomograms often include multiple parameters with the advantage to outperform specific individual variables. While numerous prognostic nomograms have been developed for bladder cancer, there has been no risk score reported for the prediction of a diagnosis of cancer in patients presenting with VH or NVH (332-334).

In this chapter, I report the development and external validation of a haematuria cancer risk score for the prediction of cancer. This will enable both patients and physicians to easily determine cancer risk following a presentation of haematuria. The advantage of a risk assessment approach over the application of arbitrary age thresholds allows for a more individualised approach, with the aim to improve detection of cancer and reduce the need for investigations in patients unlikely to

have malignancy. The findings of this chapter are now published in the *Journal of Internal Medicine* (335).

7.2 Methods

7.2.1 Study design and population

Both the development and validation cohort comprise of data from prospectively recruited patients who were referred to secondary care following a presentation of haematuria. NVH was defined as urine dipstick of ≥1+ of blood on ≥2 occasions in the discovery cohort (13). NVH was defined by either haematuria on urine dip stick or urine microscopy in the validation cohort due to the absence of haematuria guidelines in Switzerland and the variation in physician practice patterns. Patients in the development cohort were recruited between March 2016 and June 2017 at 40 UK hospitals. The external validation cohort consists of patients recruited between 2011-2017 from the Department of Urology, University of Zurich. Non-patient identifiable details and clinical variables were provided by Dr Christian Fankhauser (Urology resident, Department of Urology, University of Zurich). All patients were ≥18 years and were referred to secondary care following a presentation of haematuria in the community. Study design and patient eligibility criteria have been previously described (336).

All patients had no previous history of a bladder cancer diagnosis and evaluation comprised of medical history and clinical examination. Patient demographics, gender, ethnicity, smoking history and occupation were recorded. Occupational risk factor was defined as patients working as gardener, painter, hairdresser/ barber, textile worker or metals factory worker (337). Cystoscopy and upper tract imaging were performed for all patients. When bladder cancer was suspected, patients underwent a TURBT or bladder biopsy under general anaesthesia. Bladder tumours were defined as UCC and other bladder cancer variants

confirmed on histology. Upper tract cancers were also confirmed on histology and classified to either upper tract urothelial cancer or renal cell cancers.

The development cohort of the study received ethical approval by Health Research Authority- North West Liverpool Central Research Ethics Committee on March 2016 (IRAS project ID: 179245, REC reference: 16/NW/0150). The validation cohort received ethical approval by the cantonal ethic committee of Zurich (STV KEK-ZH BASEC-Nr. 2016-00158).

7.2.2 Development and validation of a novel haematuria cancer risk score & statistical analysis

Univariate logistic regression was used to determine an association between individual variables and bladder cancer. The outcome was bladder cancer which was defined as Yes=1 versus No=0. All cases were used for estimating odds ratios. Age (years) was analysed as a continuous variable while gender (0=female, 1=male), type of haematuria (0=NVH, 1=VH), smoking history (0=non-smoker, 1=ex-smoker, 2= current smoker, 3=missing) and Ethnicity (0=white, 1=non-white, 2=missing) as categorical variables. Multivariate logistic regression model was performed with patient's age, gender, type of haematuria and smoking history were used as the final predictors for the diagnosis of bladder cancer (0=No vs 1=Yes).

A novel haematuria cancer risk score was developed as the linear predictor of the fitted multivariate logistic regression in the derivation dataset and fitted as a single predictor to the validation dataset. To assess the performance of the novel haematuria cancer risk score, the AUC was used as a measure of discrimination.

The lower and upper 95% CI of the AUC were computed as defined by DeLong et al.(338). Venkatraman's test for two unpaired ROC was performed using 2000 resampling to test the null hypothesis that the true difference in AUC is equal to 0 (339). External validation was then performed using the Swiss patient cohort. The prediction accuracy of the novel haematuria cancer risk score was evaluated by the calibration slope in the validation dataset.

All statistical analyses were performed with R (R Foundation for Statistical Computing; version 3.4.3) (340). All applied tests were two-sided and a p value <0.05 was accepted as statistically significant. The development cohort of this study was registered with ClinicalTrials.gov: NCT02676180. Statistical supported for provided by Dr Amar S Ahmad (Biostatistician, Centre for Cancer Prevention, Queen Mary, University of London).

7.3 Results

7.3.1 Patient demographics

A total of 3539 and 656 patients were used in the development and validation cohort respectively. Descriptive patient characteristics of both study populations are shown in Table 1. Box plot stratifying patients in the development cohort according to the presence or absence of bladder cancer at histology and smoking history according to age is shown in Figure 7.1. In the development cohort, 285 patients (8.1%) had a diagnosis of bladder cancer and 69 patients (10.7%) had a diagnosis of bladder cancer in the validation cohort. The histological characteristics of bladder cancer in the development and validation cohort are shown in Table 7.1. Univariate logistic regression analysis report that older patients ($p<0.01$), patients with VH ($p<0.01$), male patients ($p<0.01$), white patients ($p=0.044$) and patients with a smoking history ($p=0.002$) were significantly more likely to have a diagnosis of bladder cancer. In our cohort, occupational history was not associated with a diagnosis of bladder cancer ($p=0.8$).

Figure 7.1: Box plot stratifying patients in the development cohort according to the presence of absence of bladder cancer at histology and smoking history according to age.

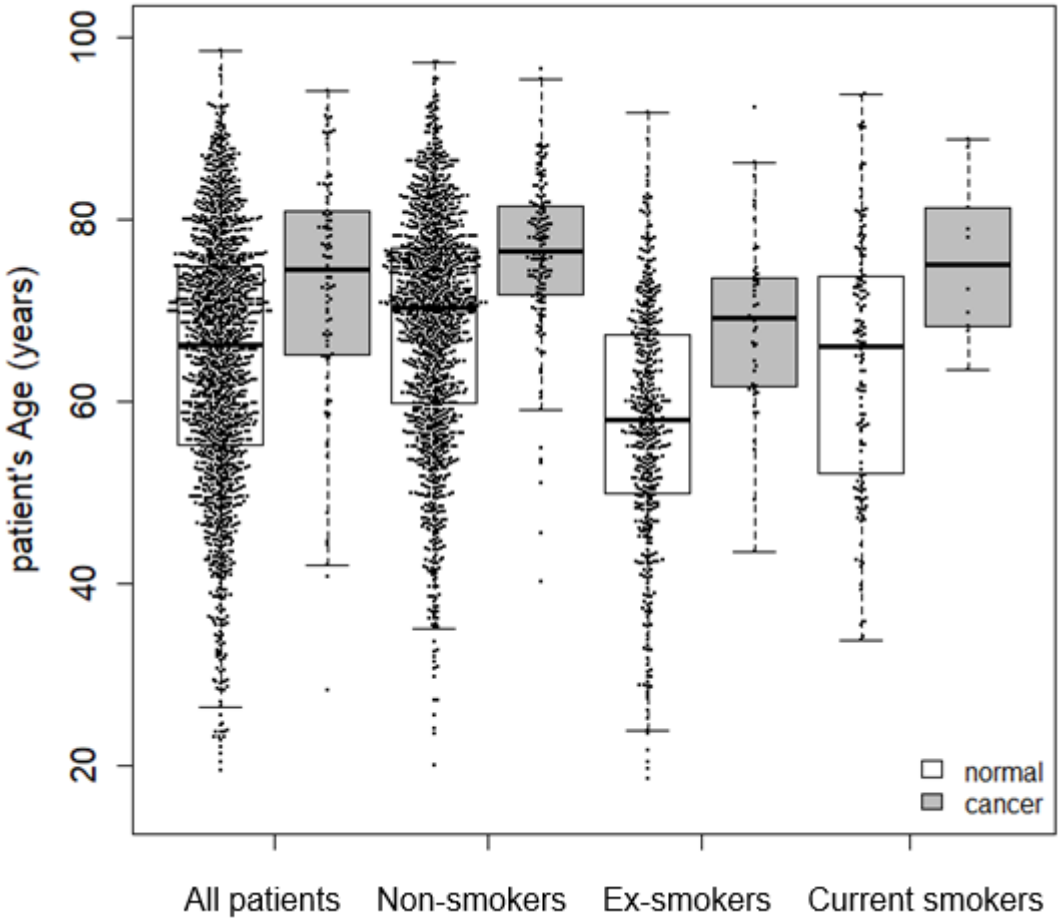


Table 7.1: Patient demographics and bladder cancer histology type, grade and stage of development and validation cohorts.

Variables	Development cohort (n=3,539)	Validation cohort (n=656)
Age (median, IQR)	68 (57, 76)	57 (47, 68)
Haematuria, n (%):		
Visible	2,296 (64.9)	322 (49.1)
Non-visible	1,243 (35.1)	334 (50.9)
Gender, n (%):		
Male	2,098 (59.3)	504 (76.8)
Female	1,441 (40.7)	152 (23.2)
Ethnicity, n (%):		
White	2,977 (93.8)	
Non-white	196 (6.2)	
Smoking history, n (%):		
Non-smoker	1,519 (44.6)	212 (32.3)
Ex-smoker	1,387 (40.7)	154 (23.5)
Current smoker	500 (14.7)	290 (44.2)
Occupational risk factor, n (%):		
Yes	529 (16.2)	
No	2,743 (83.8)	
Bladder pathology histology type, n (%):		
Bladder transitional cell carcinoma	279 (97.9)	67 (95.7)
Squamous cell carcinoma	4 (1.4)	0 (0)
Adenocarcinoma	2 (0.7)	1 (1.4)
Sarcoma	-	2 (2.9)
Bladder cancer grade, n (%):		
G1	33 (11.6)	Low grade: 11 (15.9)
G2	118 (41.4)	
G3	134 (47.0)	High grade: 58 (84.1)
Not known	-	1
Bladder cancer histology stage, n (%):		
CIS	3 (1.1)	4 (5.7)
pTa	173 (60.7)	25 (35.7)
pT1	57 (20.0)	21 (30.0)
≥pT2	52 (18.2)	20 (28.6)

7.3.2 Development and validation of the haematuria cancer risk score

Spearman's correlation between bladder cancer predictors showed that no strong correlation was observed between predictors (Table 7.2).

Table 7.2: Spearman's correlation between bladder cancer predictors.

	Age	Gender	Haematuria	Smoking	Ethnicity
Age	1	0.147	0.019	-0.050	0.145
Gender		1	0.280	0.072	-0.026
Haematuria			1	-0.011	-0.010
Smoking				1	0.070
Ethnicity					1

Multivariate logistic regression model showed that increasing age (OR 2.9, 95% CI 2.3- 3.6, p<0.001), VH (OR 3.9, 95% CI 2.6- 5.6, p<0.001), gender [Male (OR 1.8, 95% CI 1.3- 2.4, p<0.001)] and smoking history [ex-smoker (OR 1.5, 95% CI 1.1- 2.0) & current smoker (OR 2.6, 95% CI 1.7- 3.8), p<0.001] were independently associated with a bladder cancer diagnosis (Table 7.3). Patients who were ex-smokers were more at risk compared to current smokers in univariate logistic regression. However, after adjusting for age in a bivariate logistic regression model as well as in a multivariate regression model, current smokers were more at risk for bladder cancers compared to ex-smokers (p<0.01). A novel haematuria cancer risk score was developed as the linear predictor of the fitted multivariate logistic regression:

Haematuria cancer risk score = 0.055*Age + 1.348*Haematuria type + 0.576*Gender + 0.413*Ex-Smoker + 0.943* Current-Smoker

Table 7.3: Univariate and multivariate logistic regression models associated with bladder cancer in the development cohort. N=3539 (Bladder Cancer=285).

		Univariate			**Multivariate**	
Predictor	Unit	IQR-OR† (95% CI)	LR χ^2 (d.f., P)		IQR-OR† (95% CI)	$\Delta\chi^2$ (d.f., P)
Age	years	2.931 (2.377, 3.614)	120.07 (1, <2.2e-16)		2.892 (2.319, 3.605)	120.07 (1, <2.2e-16)
Haematuria	Non-visible	1 (ref)				
	Visible	4.526 (3.127, 6.551)	89.007 (1, <2.2e-16)		3.850 (2.629, 5.638)	84.119 (1, <2.2e-16)
Gender	Female	1 (ref)				
	Male	2.960 (2.196, 3.990)	60.044 (1, 9.3e-15)		1.779 (1.298, 2.438)	16.346 (1, 5.3e-05)
Smoker*	Non-smoker	1 (ref)				
	Ex-smoker	1.917 (1.453, 2.531)			1.512 (1.132, 2.018)	
	Current smoker	1.619 (1.112, 2.357)	22.638 (3, 4.8e-05)		2.568 (1.719, 3.836)	
	Missing*	1.223 (0.621, 2.410)			1.283 (0.636, 2.585)	21.723 (3, 7.4e-05)
Ethnicity**	White	1 (ref)				
	Non-White	0.490 (0.248, 0.967)	11.097 (2, 0.00389)			NSS
	Missing*	0.496 (0.274, 0.896)				
† Interquartile-range odds ratios for continuous predictors and simple odds ratios for categorical predictors.					Model LR χ^2 (d.f, P) = 242.257 (6, <2.2e-16)	
* Fourth category for Smoking 'missing' was created and compared to non-smokers category in the logistic regression analysis ** Third category for Ethnicity and compared to White category in the logistic regression analysis					Harrell's c-index = 0.768 (95%CI: 0.741, 0.795)	

Abbreviations: IQR: Interquartile Range; OR: Odds Ratios; CI: confidence interval; LR: likelihood ratio; χ^2: chi-square test (degrees of freedom, p-value); $\Delta\chi^2$: delta chi-square (degrees of freedom, p-value), terms added sequentially (first to last). NSS: Not Statistically Significant.

Figure 7.2 shows the distribution of the haematuria cancer risk score. The area under the receiver operator characteristics (ROC) curve of the haematuria cancer risk core was AUC=0.768 (95% CI 0.741- 0.795) in the development cohort and AUC= 0.835 (95% CI 0.789- 0.880) in the validation cohort (Figure 7.3). No statistically significant difference was observed (p-value= 0.1015) between AUC values of the derivation and validation datasets by Venkatraman's test with 2000 bootstraps (339). The estimated calibration slope in the validation dataset was 1.215. The slope is greater than one, but it is not significantly different to one (p=0.151) hence, the discrimination seems to be preserved.

Figure 7.2: Histogram of the haematuria cancer risk score in the development and validation datasets. The vertical solid, dashed and dotted lines show the 25th, 50th and 75th centiles of the haematuria cancer risk score in each dataset.

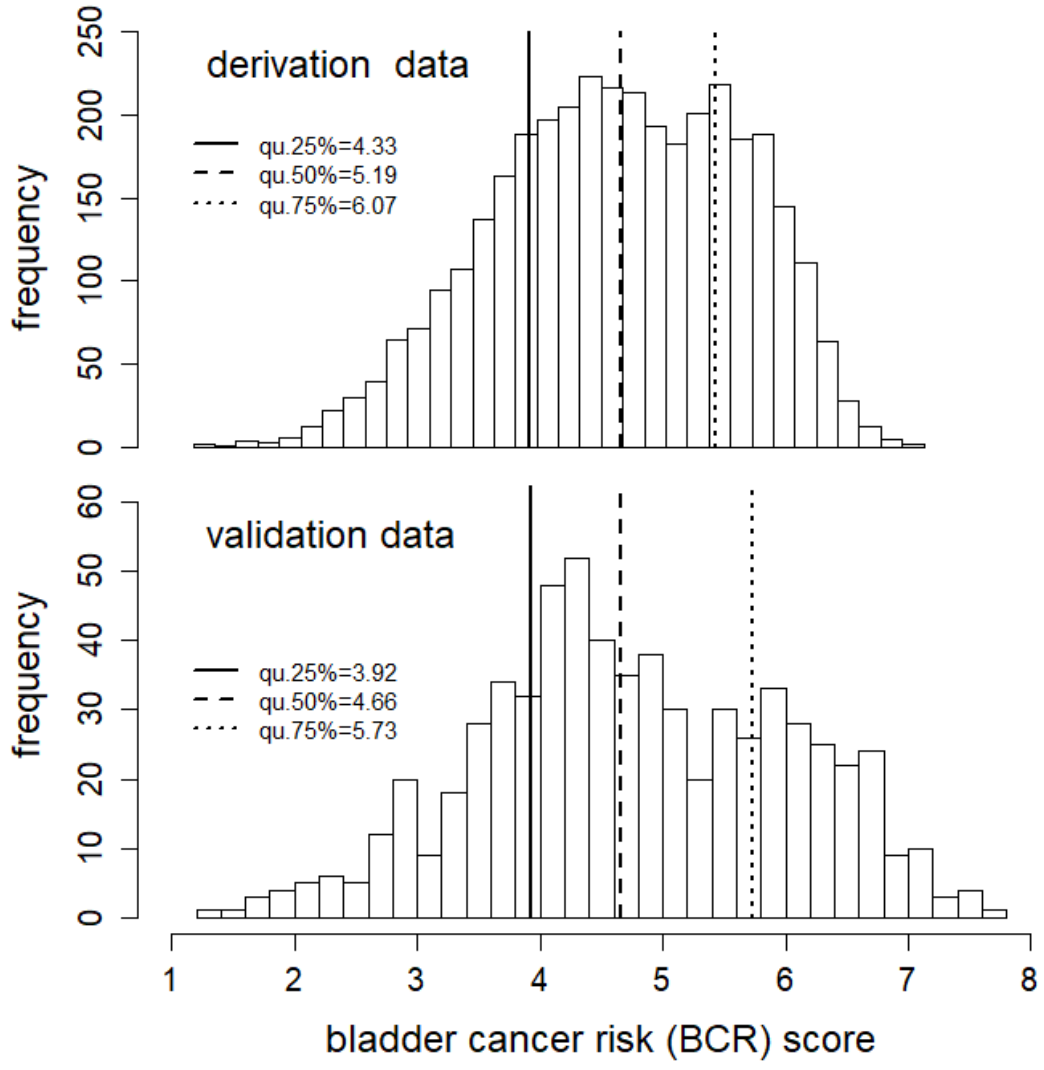
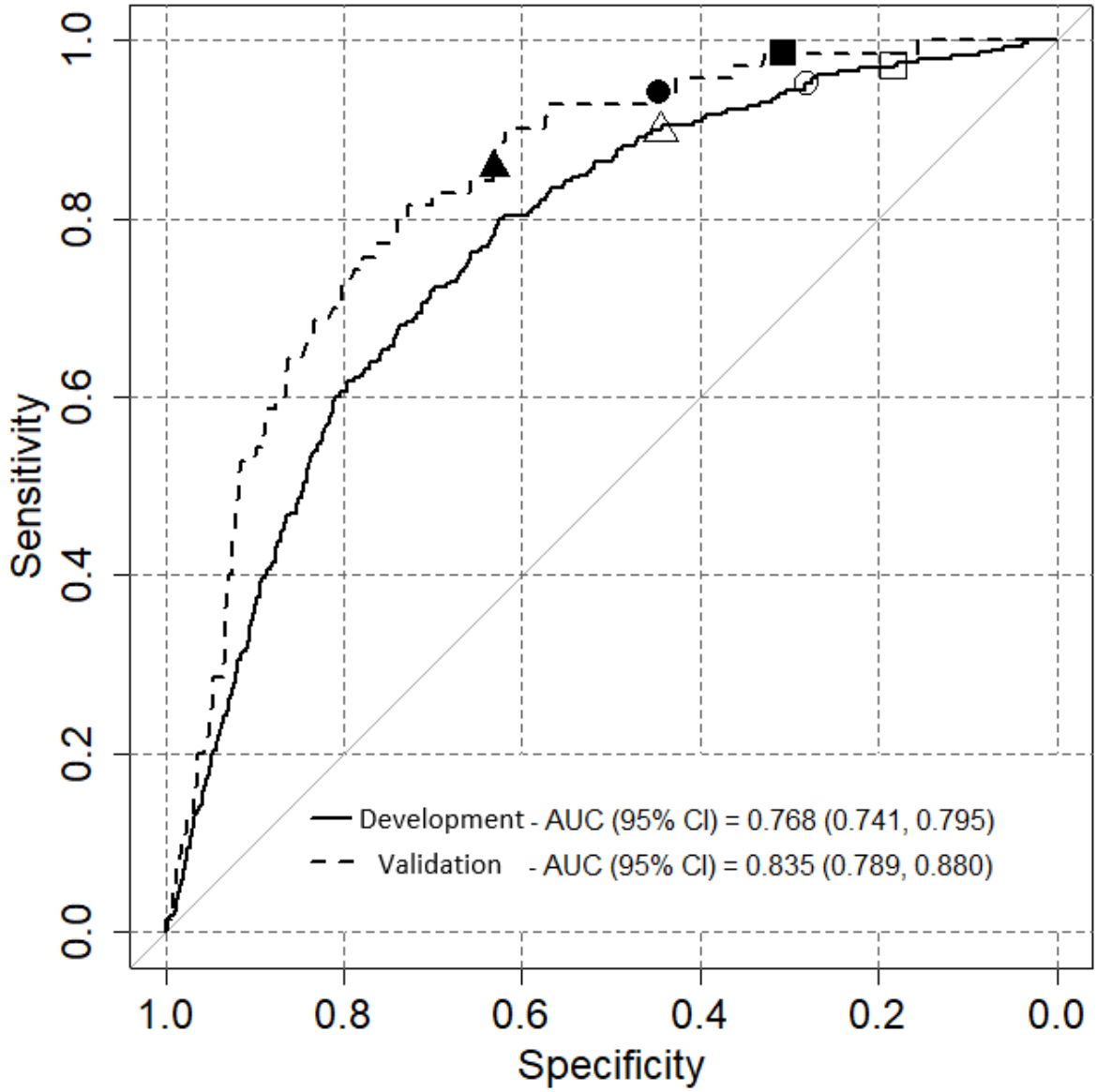


Figure 7.3: ROC curve of the haematuria cancer risk score. AUC 0.768 (95% CI: 0.741, 0.795) in the development cohort and AUC 0.835 (95% CI: 0.789, 0.880) in the validation cohort. The white square, circle and triangle gives 0.972 (95% CI: 0.954, 0.989), 0.951 (95% CI: 0.923, 0.975) and 0.898 (95 %CI: 0.863, 0.930) sensitivity in the development dataset with cut-off values of 4.015, 4.386 and 4.916 respectively. Using the same cut-off values, the black square, circle and triangle shows 0.986 (95% CI: 0.957, 1.000), 0.943 (95% CI: 0.886, 0.986) and 0.857 (95% CI: 0.771, 0.929) sensitivity in the validation dataset respectively.



7.3.3 Performance of the Haematuria Cancer Risk Score

Table 7.4 reports the corresponding sensitivity, specificity, true positives and negatives and false positives and negatives derived from the ROC curve for selected cut-off values. A bootstrap test with 2,000 replicates showed no statistically significant difference between the sensitivities of the development and validation cohorts (Table 7.5). Table 7.6 presents the estimated age cut-off for VH and NVH patients by smoking status for female and male patients to identify all cancers. Figure 7.4 illustrates the relative odds calculated from the fitted multivariate logistic regression model for male and female patients incorporating other risk factors including age, type of haematuria and smoking history. Elderly, male patients who were smokers with VH had the highest risk of having bladder cancer. Figure 7.5 shows the haematuria cancer risk score as a nomogram to guide who should be investigated for bladder cancer.

The development cohort comprised of 55 upper tract cancers (37 renal cell carcinoma, 18 UTUC) while the validation cohort had 12 upper tract cancers (9 renal cell carcinoma, 3 UTUC). All patients with upper tract cancers would have been selected to have haematuria investigations using the haematuria cancer risk score of >4.5.

Table 7.4 Haematuria cancer risk score cut offs with corresponding sensitivity, specificity, true positive and negative and false positive and negative derived from ROC curve in the development and validation cohort.

	Development cohort							Validation cohort					
Cut-off	**TP**	**FN**	**FP**	**TN**	**Specificity (95% CI)**	**Sensitivity (95% CI)**		**TP**	**FN**	**FP**	**TN**	**Specificity (95% CI)**	**Sensitivity (95% CI)**
3.240	283	2	3,058	196	0.060 (0.053, 0.069)	0.993 (0.982, 1.000)		70	0	517	69	0.118 (0.092, 0.143)	1.000 (1.000, 1.000)
3.897	279	6	2,748	506	0.156 (0.143, 0.168)	0.979 (0.961, 0.993)		69	1	428	158	0.270 (0.232, 0.306)	0.986 (0.957, 1.000)
4.015	277	8	2,656	598	0.184 (0.170, 0.198)	0.972 (0.954, 0.989)		69	1	406	180	0.305 (0.268, 0.346)	0.986 (0.957, 1.000)
4.334	274	11	2,380	874	0.269 (0.254, 0.284)	0.961 (0.937, 0.982)		67	3	336	250	0.425 (0.386, 0.468)	0.957 (0.914, 1.000)
4.386	271	14	2,337	917	0.282 (0.267, 0.298)	0.951 (0.923, 0.975)		66	4	324	262	0.445 (0.406, 0.486)	0.943 (0.886, 0.986)
4.492	268	17	2,239	1,015	0.312 (0.296, 0.329)	0.940 (0.912, 0.965)		65	5	296	290	0.494 (0.454, 0.536)	0.929 (0.871, 0.986)
4.559	265	20	2,171	1,083	0.333 (0.317, 0.349)	0.930 (0.898, 0.958)		65	5	285	301	0.512 (0.473, 0.556)	0.929 (0.871, 0.986)
4.681	263	22	2,050	1,204	0.370 (0.354, 0.387)	0.923 (0.891, 0.951)		65	5	261	325	0.555 (0.514, 0.596)	0.929 (0.871, 0.986)
4.681	262	23	2,050	1,204	0.370 (0.354, 0.387)	0.919 (0.888, 0.951)		65	5	261	325	0.555 (0.514, 0.596)	0.929 (0.871, 0.986)
4.916	256	29	1,806	1,448	0.445 (0.428, 0.462)	0.898 (0.863, 0.930)		60	10	216	370	0.631 (0.592, 0.671)	0.857 (0.771, 0.929)

TP: true positive, FN: false negative, FP: false positive, TN: true negative, 95% CI: 95% confidence interval

Table 7.5: Comparison of sensitivities of the haematuria cancer risk score in the development and validation datasets based on 2000 bootstrap replicates for the selected cut-off values in Table 7.4.

Cut-off	Development	Validation	Difference	χ^2	p-value	adjusted p value
3.240	0.993	1.000	-0.007	1.810	0.178	0.595
3.897	0.979	0.986	-0.007	0.710	0.400	0.782
4.015	0.972	0.986	-0.014	2.472	0.116	0.579
4.334	0.961	0.957	0.004	0.172	0.678	0.782
4.386	0.951	0.943	0.008	0.442	0.506	0.782
4.492	0.940	0.929	0.012	0.783	0.376	0.782
4.559	0.930	0.929	0.001	0.007	0.931	0.931
4.681	0.923	0.929	-0.006	0.144	0.704	0.782
4.681	0.919	0.929	-0.009	0.358	0.550	0.782
4.916	0.898	0.857	0.041	5.707	0.017	0.169

Table 7.6: Estimated age cut-off for referral of gross haematuria and microscopic haematuria to identify all cancers.

	Female			**Male**				**NICE**	**AUA**
	Non-Smoker	Ex-Smoker	Current-Smoker	Non-Smoker	Ex-Smoker	Current-Smoker			
NVH	73	66	56	63	55	45		60	35
VH	49	41	31	38	31	21		45	-

AUA: American Urological Association, NICE: National Institute for Health and Care Excellence, NVH: Non-visible haematuria, VH: Visible haematuria

7.7.4 Comparison between haematuria cancer risk score with existing haematuria guidelines

We explored the performance of the HCRS using a defined cut-off of 4.015, where patients with a HCRS of ≥4.015 should have investigations following a presentation of haematuria. This was based on a sensitivity of approximately 97% for all cancers. I then tested the haematuria cancer risk score in the Swiss external validation cohort. In the validation cohort, referral for investigation of haematuria based on NICE guidance would miss 12.9% (n=9) of all urinary tract

cancers (6 bladder cancers, 3 renal cell cancers) reporting a sensitivity of 87.1%. Applying the NICE guideline criteria, 268 patients were true negative cases and 318 patients were false positive cases equating to a specificity of 45.7%. The AUA recommendation for the investigation of haematuria had a sensitivity of 100% with 80 true negative patients and 555 false negative patients corresponding to a specificity of 12.6%.

By comparison, using the same the haematuria cancer risk score threshold (4.015), a sensitivity of 98.6% was achieved with a corresponding specificity of 30.5% suggesting that an additional 11.4% (n=8) of urinary tract cancers were detected which would have been missed when applying the NICE guidance. The haematuria cancer risk score missed one bladder cancer but reduce the number of patients requiring investigations by 149 patients.

The American Urological Association guidelines for haematuria would identify all cancers but result in a specificity of 3.6% compared to the 30.5% achieved using the risk score approach. All patients with upper tract cancers would have been referred for investigation.

Figure 7.4: Estimated probability of bladder cancer by age, type of haematuria and smoking history for male (A) and female (B).

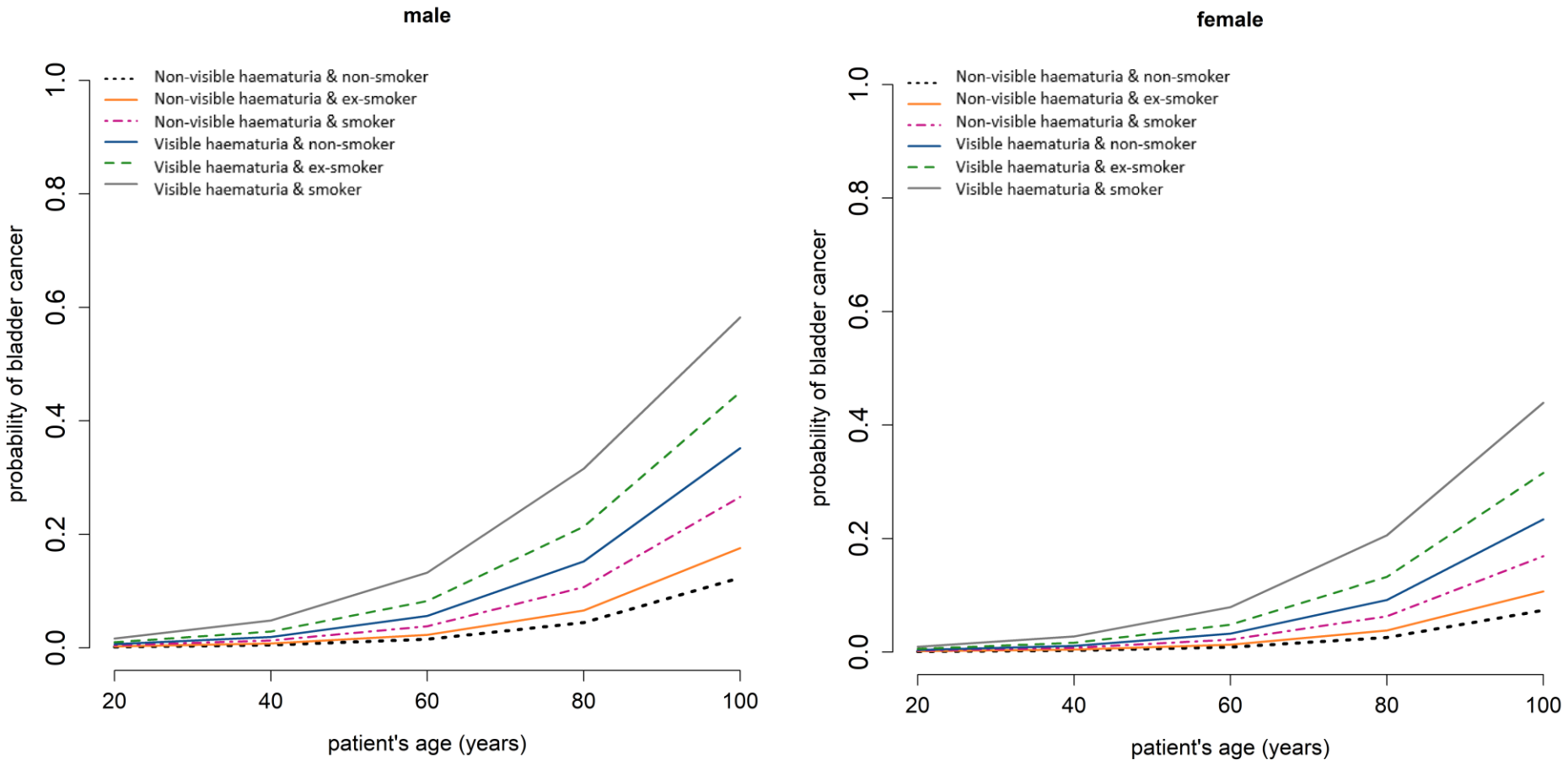
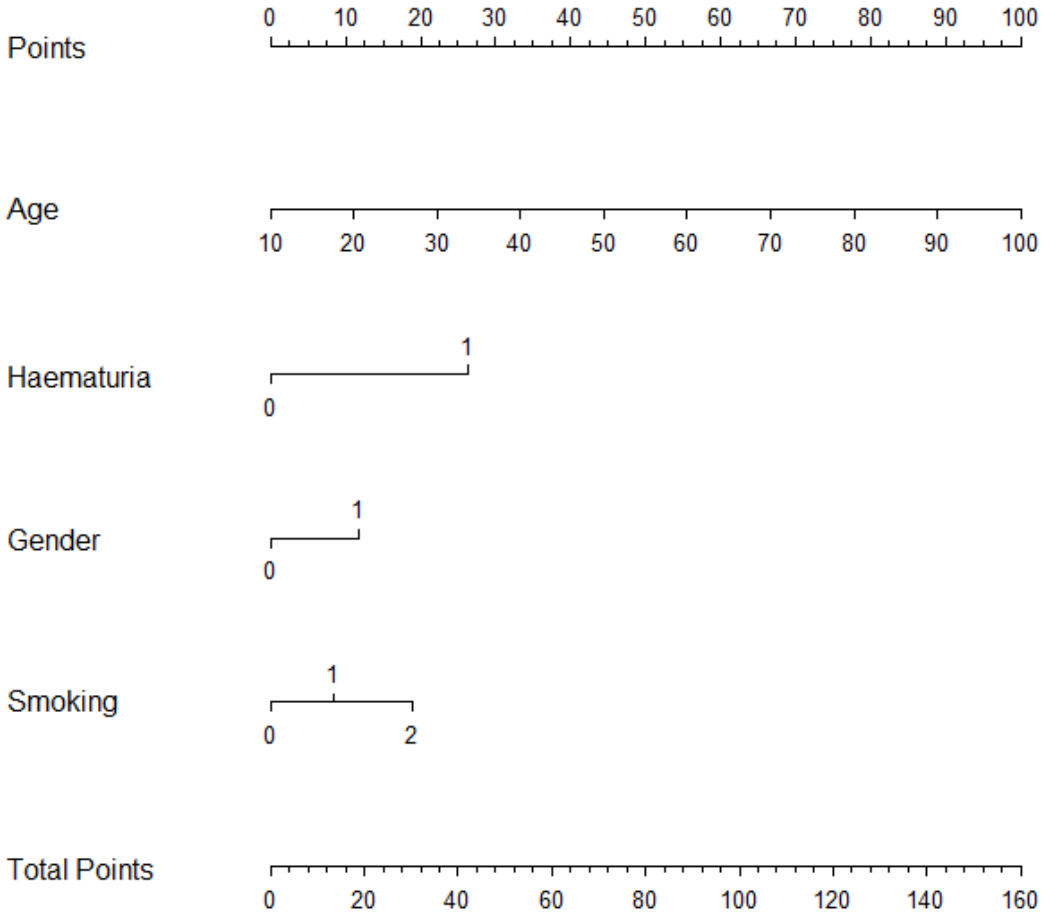


Figure 7.5: Nomogram to guide who should be investigated for cancer following a presentation of haematuria. Each predictor with a given value can be mapped to the Points axis. The sum of these points can be referred to in the Total Points axis.



7.4 Discussion

This study represents the first report describing the development and external validation of a haematuria cancer risk score to determine the risk of urinary tract cancer in patients with VH and NVH. With the assistance of Dr Amar Ahmad, I constructed the haematuria cancer risk score using patients from a prospective multi-centre observational study, thus allowing generalizability for the UK. The score was then validated using a prospective collected patient cohort referred for investigation of haematuria in Switzerland. I show that adopting a risk score approach identified significantly more urinary tract cancers (11.4%) than would have identified using the current NICE guidance and reduce the number of patients subjected to investigations compared to AUA guidance.

This study has several strengths in its methodology, patient cohort, ease of use and practical applicability to real world clinical practice. We used a reasonable sample size of 3,539 patients to derive the haematuria cancer risk score. Our model had a good discriminatory ability in the validation dataset with an AUC of 0.835 (95% CI 0.789- 0.880) which was higher in comparison to over 60% of prediction risk scores developed by Memorial Sloan Kettering Cancer Centre (MSKCC) which have an AUC of <0.750 (341).

The prospective multi-centre nature of the development cohort allows for accurate data capture by comparison to most risk prediction scores which are derived from retrospective studies or population datasets (333, 334). External validation using a patient cohort from a different country confirms the risk score is robust and reproducible Finally, variables chosen represent clinical details which are part of the standard referral criteria for suspected cancer following a

presentation of haematuria. Hence, adopting the HCRS would be straight forward without additional time pressures.

Loo and colleagues used electronic medical records (EMR) from Kaiser Permanente to identify patients who had investigations for NVH to derive a development cohort of 1973 patients and a validation cohort of 657 patients (342). Following multivariable logistic regression, they incorporated the following variables in their Haematuria Risk Index: history of VH within the last 6 months, patient age ≥50 years, history of smoking, male gender, and >25 RBS/ HPF on urine microscopy. These five variables resulted in an AUC of 0.829 in the validation cohort. The current study which assesses both VH and NVH patients achieves a similar diagnostic performance using fewer variables. Although in theory a prospective study, the quality of data recorded in an EMR can vary in quality. Another confounder was that both the development and validation cohorts were derived from the same EMR (343). One of the variables used in the study reported by Loo et al., was history of VH within the last 6 months and I would argue that these patients were evaluated for VH rather than NVH (342). Further, in the development cohort, patients who did not have complete haematuria investigations were excluded and this may introduce case selection bias. The risk score of the current study could not be compared to that of Loo et al. as different variables were utilised (342).

Another risk score developed by Wu and colleagues was designed to predict the risk of developing bladder cancer based on a case control study of 678 patients (344). The risk score did not have external validation and incorporated clinical variables such as smoking history and environmental exposure to carcinogens to achieve an AUC of 0.70 (95% CI 0.67-0.73). Incorporating mutagen sensitivity

data increased the AUC to 0.80 (95% 0.72-0.82). The risk score by Wu and colleagues was developed to identify patients at risk of developing future bladder cancers who may benefit from screening (344).

Although bladder cancer is predominantly a disease of older men with a median age of 70 years, it is not uncommon for younger patients to be diagnosed with bladder cancer (55). Recommendations excluding younger patients (<60 years with NVH and <45 years with VH) will result in missed cancers (143). Age is the main discriminating factor across guidelines and we show that the usage of the haematuria cancer risk score overcomes this limitation. Early detection of cancer is a cornerstone of the NHS cancer strategy as late presentation of bladder cancer is associated with reduced overall survival (345). However, 18% of patients diagnosed with bladder cancer consult their general practitioner ≥3 times prior to referral for investigations which suggests the need for less restrictive recommendations to enable prompt referral for investigations (148).

In addition to age and type of haematuria, smoking history and gender are important risk factors for bladder cancer (26, 55). These variables are not currently used in the decision to refer for investigations but are routinely collected as part of the standard assessment of patients. I have shown that incorporating all four variables in a risk assessment approach would improve the patient selection for haematuria investigations compared to current referral based on age thresholds and type of haematuria alone.

7.5 Limitations

There are several limitations in this study. The development cohort reflects a UK haematuria referral pattern and although validated in a Swiss population, further testing in non-European countries should be considered before use. As discussed previously, recent NICE guidance recommend referral for investigations of patients with VH aged ≥45 years and ≥60 for patients with NVH. Hence, there may be case selection for patients who were investigated although 16.9% of patients investigated for haematuria were below these age thresholds. Patients were recruited in secondary care and although guidelines for referral exist to aid primary care decision making, it is possible that a case selection bias exists whereby not all patients presenting with haematuria in primary care are referred for investigations according to existing guidelines. The development of a risk assessment tool was not a pre-planned analysis hence I was limited by the variables that I could use.

7.6 Conclusions

In this chapter, I report the development and external validation of the first haematuria cancer risk score to identify patients with VH and NVH who are at risk of harbouring cancer. The haematuria cancer risk score improves cancer detection rate and performs better than existing criteria to trigger referral for haematuria investigations. Further validation would be useful to confirm the generalisability of this haematuria cancer risk score to other countries.

CHAPTER 8 : PATIENT PERSPECTIVES ON CYSTOSCOPY AND THE USE OF URINARY TEST TO DETECT BLADDER CANCER IN THE SURVEILLANCE SETTING

8.1 Introduction

NMIBC accounts for over 75% of all new bladder cancer cases diagnosed and has a 28-50% risk of recurrence and 5-20% risk of progression at 5 years (86). The risk of recurrence necessities regular surveillance cystoscopy and guidelines recommend a risk adapted approach which can be as frequent as three monthly cystoscopy with lifelong follow-up for high risk disease (87). The requirement for vigilant surveillance strategies is responsible for the high cost of healthcare associated with bladder cancer (346).

The development of flexible cystoscopy has led to the widespread use of cystoscopy performed under local anaesthetic in the outpatient setting. However, despite this, flexible cystoscopy remains an invasive procedure with associated patient discomfort. Further, data from primary care suggest that up to 5% of patients develop UTI following cystoscopy (20). The requirement for life long regular surveillance cystoscopy has profound direct healthcare related cost making bladder cancer one of the most expansive cancers to manage (347).

The potential for urinary biomarkers for the detection of bladder cancer is an area of active research and several biomarkers have been approved for use. Commercially available tests are licensed only as companion test as they do not have the required diagnostic performance to replace cystoscopy (sensitivities of 57-82%) (131). A systematic review performed in Chapter 5 highlights promising biomarker panels, however, they remain unvalidated and further prospective clinical trials are required. Hence, before implementing new technology in clinical practice, it is essential to understand patients' acceptance and requirement for such a test.

To understand patient views relating to cystoscopy and the potential to integrate urinary based biomarkers in a surveillance programme, I assessed the minimal accepted sensitivity (MAS) of a urinary biomarker that patients with NMIBC are willing to accept as a replacement for cystoscopy in the DETECT II patient cohort using a mixed method analysis. I also report patients' experience and adverse events during and following flexible cystoscopy assessed using a patient questionnaire. Qualitative analysis by semi-structured interviews was used to explore reasons for their preference. For the first time, I also accessed the cognitive and emotional state of NMIBC patients using the Brief Illness Perception Questionnaire (BIPQ). Results of this chapter have been published in the *British Journal of Urology International* and was selected as best poster at the *European Association of Urology* 2019 annual meeting under the Urine, serum and tissue diagnostic innovations in urothelial cancer session (348).

8.2 Methods

8.2.1 Patient selection

Between September 2016- April 2017, a total of 370 patients with histologically confirmed NMIBC and a minimum of 6 months (2 surveillance cystoscopies and 2 urine collection for biomarker testing) follow-up were recruited from 52 UK hospitals. Patients were sent a patient questionnaire by post which included a brief illness perception questionnaire (BIPQ).

A sub-group of 20 English speaking patients from this cohort consented to participate in a semi-structured telephone interview. Twenty percent of patients interviewed were low grade cancers to ensure adequate representation of NMIBC.

8.2.2 Assessments

8.2.2.1 Patient questionnaire

A medical history and clinical examination were performed on all patients. Patient demographics, highest education level attained, and previous history of bladder cancer were recorded. Clinical stage was assessed using TNM WHO cancer classification (73). Cancer risk was assessed using the European Association of Urology (EAU) risk classification according to clinical-pathological features (25). Distance from patients' home to local hospital by private transport were assessed using www.maps.google.com

I constructed a patient experience questionnaire following consultation with the Dr Malgorzata Heinrich (Health Behaviour Research Centre, UCL) as no validated questionnaire exists. Patients completed a self-directed questionnaire

which was posted back to the clinical trial unit (Appendix A13). Domains assessed overall experience of cystoscopy, anxiety preceding cystoscopy and pain experienced using a 5-point Likert-scale. Patients' preference for cystoscopy vs urinary biomarker were assessed using the standard gamble method (349). Standard gamble method is typically used to measure individuals' preference where uncertainty exists and individuals are allowed to express their preference based on a variety of utility values to evaluate clinical decision making. In this setting, patients are offered a hypothetical choice of a non-invasive urinary biomarker with varying sensitivity compared to cystoscopy which is sensitive but has a risk of adverse events. Patients completed a series of questions which assessed their preference with a gradual increase in sensitivity of the proposed urinary biomarker. Cystoscopy was defined as having a sensitivity of 98% (133). The MAS for a urinary biomarker was defined as the sensitivity at which patients expressed either a preference for a urine biomarker or were neutral about accepting either the biomarker or cystoscopy.

The BIPQ completed by patients was used to assess the cognitive and emotional state of patients (350). Five of the questions assess cognitive illness representations: (Q1) How much does your illness affect your life (consequences); (Q2) How long do you think your illness will continue (timeline); (Q3) How much control do you feel you have over your illness (personal control); (Q4) How much do you think your treatment can help your illness (treatment control); and (Q5) How much do you experience symptoms from your illness (identity). Two questions assess emotional representations: (Q6) How concerned are you about your illness (concern) and (Q7) How much does your illness affect you emotionally (emotional representation). One question assesses illness comprehensibility: (Q8) How well do you understand your illness (coherence).

To compute the overall score, answer scales of three items (personal control (Q3), treatment control (Q4) and coherence (Q7)) were reversed and the sum for all eight questions were calculated, where a higher score would imply a worse illness perception. The complete questionnaire is shown in Appendix A13.

8.2.2.2 Qualitative semi-structured interview

Each qualitative semi-structured interview lasted between 20-40 minutes. All interviews were carried out by the same interviewer. Patients were interviewed after a minimum of 6 months' follow-up to ensure all patients will be able to provide an informed opinion after having both diagnostic investigations cystoscopy and urine-based test. The interview was designed to assess the following:

- Experience of cystoscopy
- Perceived advantages and disadvantages of cystoscopy
- Perceived advantages and disadvantages of urine biomarker
- Reasons for the preference for cystoscopy or urine biomarker
- Perceived acceptable sensitivity of a urine biomarker for detection of bladder cancer
- Preference for a surveillance pathway combining a urine biomarker interspaced with cystoscopy

The semi-structured interview guide is shown in Appendix A14 and was developed in collaboration with Prof Chirk Jenn Ng (Department of Primary Care, University of Malaya, Kuala Lumpur, Malaysia).

8.2.3 Data analysis

8.2.3.1 Observational study

Continuous data were reported using descriptive statistics such as mean, median, interquartile range and 95% confidence interval. Categorical variables were compared using Chi-square test. T-test and ANOVA were used to compare the mean of continuous variables. Missing data were reported as not known. SPSS v22 (IBM Corp, Armonk, New York, USA) was used for statistical analysis. All statistical tests were 2-sided and statistical significance was set at $p<0.05$.

8.2.3.2 Qualitative study

Patient interviews were performed using Skype (Microsoft, Redmond, WA, USA) and Evaer (USA) was used to record the interview. Interview recordings were transcribed, and data was managed using Nvivo 11 (QSR International, Melbourne, Australia). A thematic approach was used. Open coding was performed by two researchers on the first two transcripts and differences were resolved by discussion. Codes were assigned to sentences/ paragraphs of transcripts based on the study objective. Axial coding was performed, and existing codes combined to create larger themes. One researcher continued to code remaining transcripts and any new emerging codes were discussed. Comparisons were made throughout the analysis to form the final framework. Background notes throughout all study phases were reflected to avoid potential bias in the results.

8.2.4 Power calculations and sample size

For the patient questionnaire study, assuming a population of 10,000 new bladder cancer diagnosed in the UK each year and a 7% margin of error, a sample size of 193 patients were required to ensure a valid result with a 95% confidence

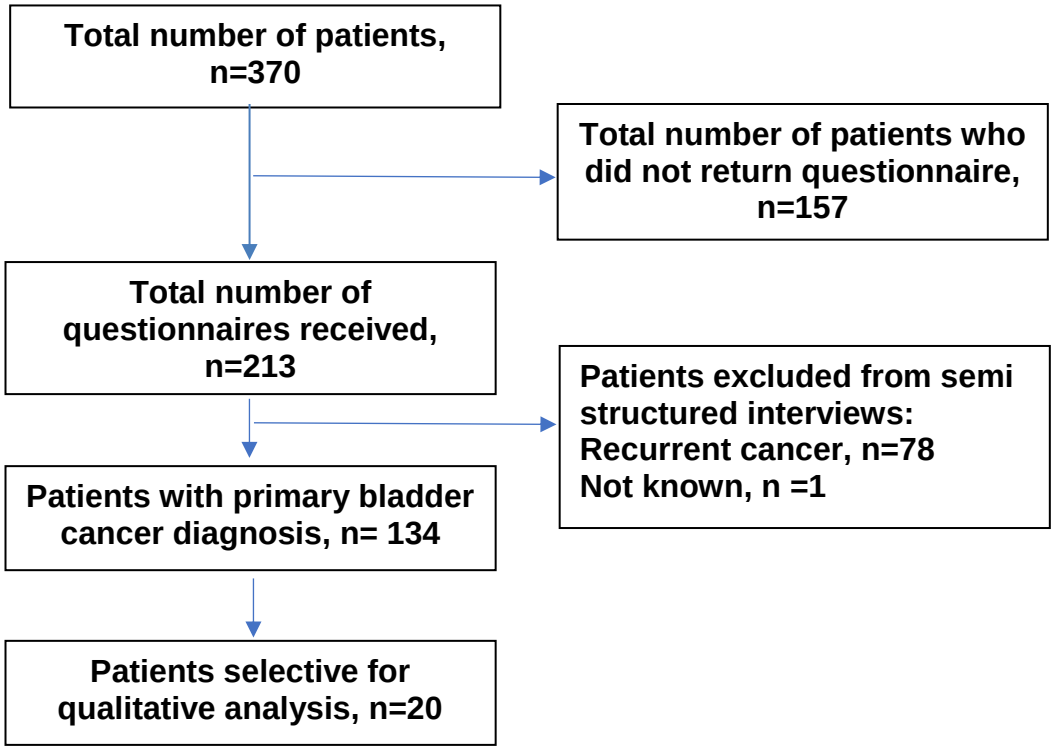
interval. A total of 20 patients were selected for the semi-structured interviews for qualitative analysis which is an established figure for qualitative analysis. It is expected that interviewing beyond 20 subjects would elicit in similar responses.

8.3 Results

8.3.1 Patient cohort

At the time of data analysis, 370 patients had histologically confirmed NMIBC with at least 6 months follow up. A total of 157 patients did not return the questionnaire mailed to them suggesting a response rate of 57.6%. Twenty consecutive patients with a new diagnosis of bladder cancer who consented for the qualitative sub-study were interviewed. A flow diagram of patients included for analysis is shown in Figure 8.1.

Figure 8.1: Flow diagram of patients in questionnaire and qualitative study.



8.3.1.1 Patient questionnaire

8.3.1.1.1 Patient demographics

Baseline characteristics and clinical-pathological variables for 213 patients are shown in Table 8.1. Median age was 74.0 years and 167 (78.4%) patients were male. Seventy nine (37.1%) patients had a history of bladder cancer prior to study enrolment. A total of 158 (74.1%) patients had ≤5 previous cystoscopies. Histologically defined high risk cancer according to EAU classification was confirmed in 83 (40.3%) patients. However, only 38 (17.8%) patients believed that they have bladder cancer with a high risk of recurrence.

8.3.1.1.2 Patient reported adverse events

Majority of patients reported an adverse event following cystoscopy, with 165 (77.5%) of patients reporting at least one adverse event (Table 8.2). Haematuria, dysuria or LUTS and UTI requiring antibiotics were self-reported in 100 (46.9%), 143 (67.1%) and 51 (23.1%) patients respectively. Overall, 13.4% of patients reported moderate to severe symptoms following cystoscopy (Figure 8.2). Moderate to significant pain during cystoscopy was reported in 11.5% of patients while 10.0% of patients reported moderate to significant anxiety preceding cystoscopy.

Table 8.1: Patient demographics and clinical-pathological variables

Variable	N=213
Age, median (IQR)	74.0 (67.1- 81.1)
Gender, n (%) **Male**	 170 (79.8)
Highest education, n (%) **No formal education** **High school** **GCSE** **A-levels**	 8 (3.8) 56 (26.3) 39 (18.3) 20 (9.4)

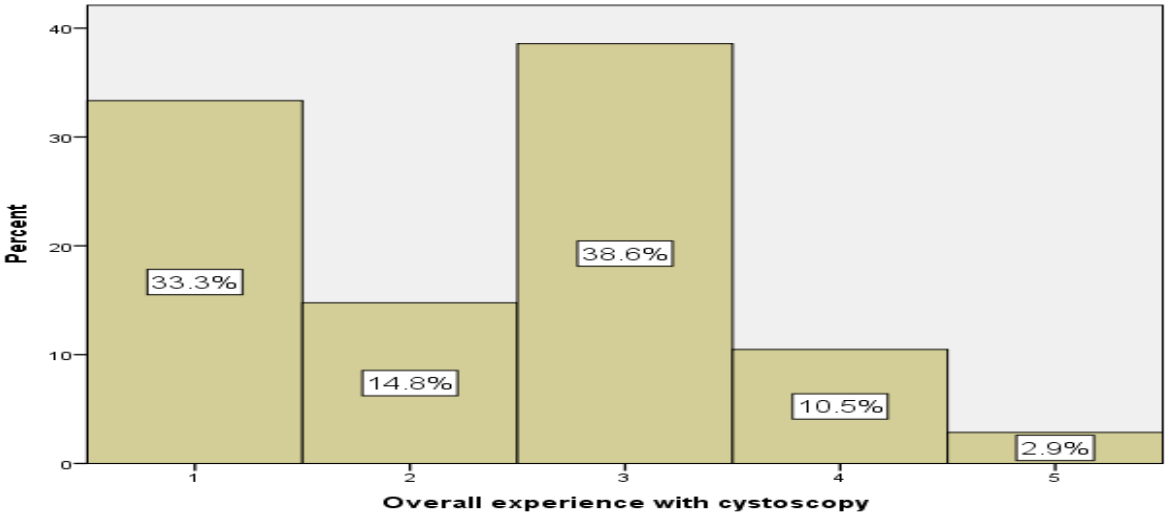
University or higher degree	31 (14.6)
Not known	59 (27.7)
Smoking history, n (%)	
Non-smoker	56 (26.3)
Ex-smoker	129 (60.6)
Current smoker	18 (8.5)
Not known	10 (4.7)
Ethnicity, n (%)	
White	188 (88.3)
Non-white	6 (2.8)
Not known	19 (8.9)
Employment, n (%)	
Full time/ part-time/ home maker/ voluntary	45 (21.1)
Retired	161 (75.6)
Disability/ unemployed	4 (1.9)
Missing	3 (1.4)
New or recurrent tumour, n (%)	
New	135 (63.4)
Recurrence	78 (36.6)
Procedure, n (%)	
TURBT/ bladder biopsy	206 (96.7)
Cystodiathermy	7 (3.3)
Previous cystoscopies, n (%)	
≤2	66 (31.0)
2-5	92 (43.2)
≥6	47 (22.1)
Not known	8 (3.8)
Tumour grade, n (%)	
G1	36 (16.9)
G2	99 (46.5)
G3	71 (33.3)
Not known	7 (3.3)
Tumour stage, n (%)	
CIS	3 (1.4)
pTa	156 (73.2)
pT1	47 (22.1)
Not known	7 (3.3)
Papillary with concurrent CIS, n (%)	5 (2.4)
Disease risk, n (%)	
Low	18 (8.5)
Intermediate	105 (49.3)
High	83 (39.0)
Not known	7 (3.3)
Patients' perception of disease risk, n (%)	
Low	49 (23.0)
Intermediate	112 (52.6)
High	38 (17.8)
Not known	14 (6.6)

Table 8.2: Complications experienced following cystoscopy.

Adverse event	N (%)
Any adverse event	
Yes	165 (77.5)
No	47 (22.1)
Not known	1 (0.4)
Haematuria	
Yes	100 (46.9)
No	96 (45.1)
Not known	17 (8.0)
Dysuria/ urinary symptoms	
Yes	143 (67.1)
No	64 (30.1)
Not known	6 (2.8)
UTI requiring antibiotics	
Yes	51 (23.9)
No	147 (69.1)
Not known	15 (7.0)

Figure 8.2: Patients experience following cystoscopy: A) overall experience, B) pain during cystoscopy, C) anxiety preceding cystoscopy. 1 denotes no symptoms/ painless/ not anxious. 5 denotes severe symptoms/ very painful/ very anxious

A



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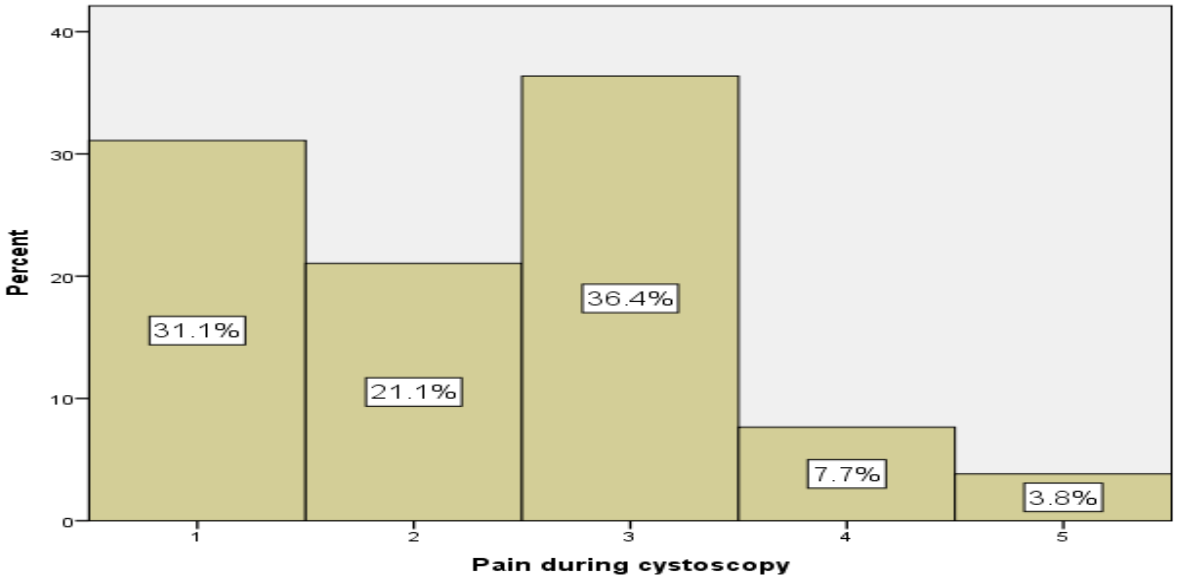
Percent
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2
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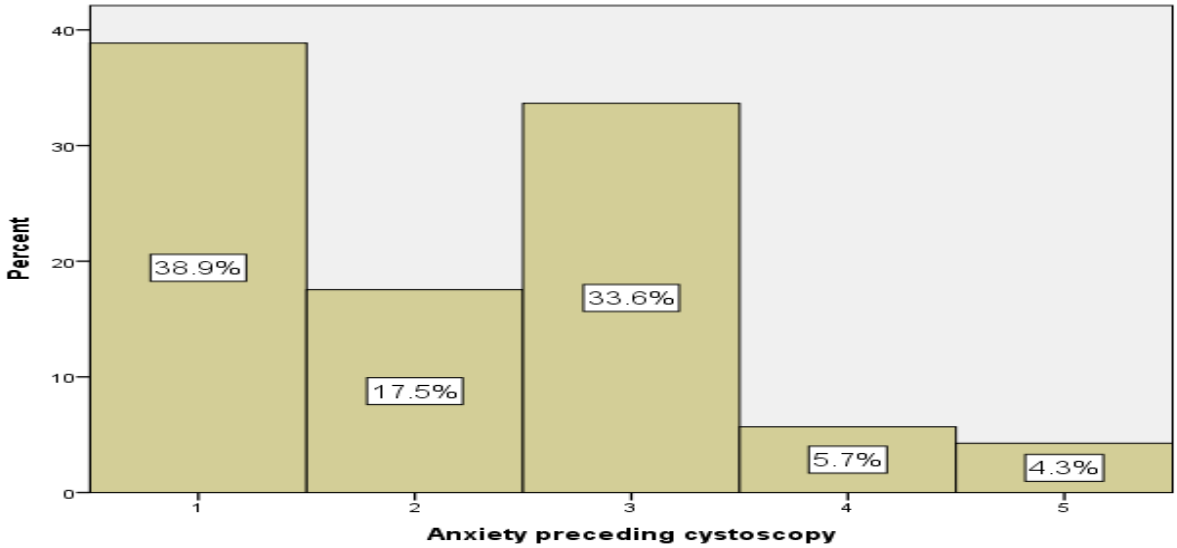
4
5
Pain during cystoscopy

C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38.9%
17.5%
33.6%
5.7%
4.3%
1
2
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4
5
Anxiety preceding cystoscopy

8.3.1.1.3 Minimum acceptable sensitivity for a urinary biomarker to replace cystoscopy

A total of 50 (24.2%) patients were prepared to accept a urinary biomarker with a sensitivity of 85% while 163 (78.7%) patients would be happy to use a urinary biomarker if it was as sensitive as cystoscopy. However, 158 (74.2%) patients would accept a urine test interspaced with cystoscopy with the view to delay the intervals between check cystoscopies (Table 8.3).

Table 8.3: Minimal acceptable sensitivity for acceptance of urinary biomarker.

Minimal acceptable sensitivity (%)	N (%)
85	50 (24.2)
90-95	26 (12.5)
96-97	30 (14.5)
98	57 (27.5)
Preference for cystoscopy even when urine test has a similar accuracy to cystoscopy	44 (21.3)
Not known	6

There was no difference in minimum acceptable sensitivity with patient demographics, previous adverse events or experience during or following cystoscopy, cancer characteristics as well as distance to hospital from patients' homes (Table 8.4). However, there was a trend towards significance where patients without recurrence <6 months (p=0.078) and male patients (p=0.052) were more likely to have a lower MAS.

Patients' confidence in the sensitivity of cystoscopy was the most common reason for choosing cystoscopy (70.5%) followed by reassurance by a clinician following cystoscopy (51.9%) and preference for diagnostic test performed at hospital (51.4%) (Table 8.5). The top reason for choosing a urinary biomarker was previous discomfort following cystoscopy (30.5%) followed by avoiding a

hospital visit (28.6%) and the non-invasive nature of a urinary biomarker test (28.1%) (Table 8.5).

Table 8.4: Patient demographics and clinical-pathological variables stratified according to minimal acceptable sensitivity.

Variables	Minimal acceptable sensitivity (%)					p value
	85	90-95	96-67	98	Cystoscopy regardless	
Median age (IQR)	73.4 (67.9-81.9)	71.6 (66.0-78.1)	72.7 (64.7-80.7)	75.5 (65.5-79.8)	76.9 (69.5-83.1)	0.486
Gender: **Male**	47 (94.0)	19 (73.1)	21 (70.0)	45 (78.9)	33 (75.0)	0.052
Highest education **No formal education** **High school** **GCSE** **A-levels** **University or higher degree** **Not known**	0 (0) 14 (28.0) 6 (12.0) 4 (8.0) 8 (16.0) 18 (36.0)	1 (3.8) 6 (23.1) 4 (15.4) 5 (19.2) 7 26.9) 3 (11.5)	1 (3.3) 11 (36.7) 5 (16.7) 3 (10.0) 2 (6.7) 8 (26.7)	4 (7.0) 13 (22.8) 16 (28.1) 4 (7.0) 9 (15.8) 11 (19.3)	2 (4.5) 12 (27.1) 7 (15.9) 2 (4.5) 4 (9.1) 17 (38.6)	0.165
Previous cystoscopies, n (%) **≤2** **2-5** **≥6** **Not known**	15 (30.0) 17 (34.0) 15 (30.0) 3 (6.0)	7 (26.9) 16 (61.5) 3 (11.5) 0 (0)	16 (53.3) 8 (26.7) 6 (20.0) 0 (0)	14 (24.6) 30 (52.6) 11 (19.3) 2 (3.5)	12 (27.3) 20 (45.5) 10 (22.7) 2 (4.5)	0.114
New or recurrent cancer **New** **Recurrent**	28 (58.0) 21 (42.0)	19 (73.1) 7 (26.9)	21 (70.0) 9 (30.0)	32 (56.1) 25 (43.9)	30 (68.2) 14 (31.8)	0.411
Previous recurrence within 6 months **Yes** **No** **Not known**	30 (60.0) 10 (20.0) 10 (20.0)	20 (76.9) 4 (15.4) 2 (7.7)	17 (56.7) 11 (36.7) 2 (6.7)	39 (68.4) 13 (22.8) 5 (8.8)	28 (63.6) 15 (34.1) 1 (2.3)	0.078
Tumour grade **G1** **G2** **G3** **Not known**	9 (18.0) 24 (48.0) 15 (30.0) 2 (4.0)	8 (30.8) 7 (26.9) 11 (42.3) 0 (0)	5 (16.7) 18 (60.0) 6 (20.0) 1 (3.3)	9 (15.8) 29 (50.9) 16 (28.1) 3 (5.3)	5 (11.4) 17 (38.6) 21 (47.7) 1 (2.3)	0.231

Tumour stage						0.428
CIS	2 (4.0)	0 (0)	0 (0)	1 (1.8)	0 (0)	
pTa	40 (80.0)	17 (65.4)	21 (70.0)	43 (75.4)	30 (68.2)	
pT1	6 (12.0)	9 (34.6)	8 (26.7)	10 (17.5)	13 (29.5)	
Not known	2 (4.0)	0 (0)	1 (3.3)	3 (5.3)	1 (2.3)	
Actual disease risk						0.482
Low	5 (10.0)	4 (15.4)	2 (6.7)	5 (8.8)	2 (4.5)	
Intermediate	26 (52.0)	9 (34.9)	18 (60.0)	31 (54.4)	18 (40.9)	
High	17 (34.0)	13 (50.0)	9 (30.0)	18 (31.6)	23 (52.3)	
Not known	2 (4.0)	0 (0)	1 (3.3)	3 (5.3)	1 (2.3)	
Patient's presumed disease risk						0.353
Low	16 32.0)	3 (11.5)	5 (16.7)	9 (15.8)	14 (31.8)	
Intermediate	22 (44.0)	14 (53.8)	18 (60.0)	34 (59.6)	24 (54.5)	
High	9 (18.0)	8 (30.8)	5 (16.7)	12 (21.1)	4 (9.1)	
Not known	3 (6.0)	1 (3.8)	2 (6.7)	2 (3.5)	2 (4.5)	
Any adverse event						0.504
Yes	41 (82.0)	17 (65.4)	23 (76.7)	45 (78.9)	36 (81.8)	
No	9 (18.0)	9 (34.6)	7 (23.3)	12 (21.1)	8 (18.2)	
Haematuria						0.484
Yes	28 (56.0)	11 (42.3)	12 (40.0)	24 (42.1)	22 (50.0)	
No	20 (40.0)	14 (53.8)	14 (46.7)	30 (52.6)	17 (38.6)	
Not known	2 (4.0)	1 (3.8)	4 (13.3)	3 (5.3)	6 (11.4)	
Dysuria/ LUTS						0.764
Yes	33 (66.0)	17 (65.4)	19 (63.3)	39 (68.4)	32 (72.7)	
No	17 (34.0)	9 (34.6)	10 (33.3)	17 (29.8)	10 (22.7)	
Not known	0 (0)	0 (0)	1 (3.3)	1 (1.8)	2 (4.5)	
UTI requiring antibiotics						0.196
Yes	9 (18.0)	4 (15.4)	10 (33.3)	13 (22.8)	13 (29.5)	
No	39 (78.0)	19 (73.1)	19 (63.3)	42 (73.7)	25 (56.8)	
Not known	2 (4.0)	3 (11.5)	1 (3.3)	2 (3.5)	6 (13.6)	
Overall experience						0.833
No symptoms	23 (46.0)	11 (42.3)	12 (41.4)	31 (54.4)	22 (51.2)	
Neutral	20 (40.0)	9 (34.6)	13 (44.8)	19 (33.3)	17 (39.5)	
Severe symptoms	7 (14.0)	6 (23.1)	4 (13.8)	7 (12.3)	4 (9.3)	

Pain						0.820
Painless	24 (48.0)	13 (50.0)	15 (50.0)	27 (47.4)	27 (61.4)	
Some what painless	19 (38.0)	9 (34.6)	11 (36.7)	22 (38.6)	15 (34.1)	
Very painful	5 (10.0)	4 (15.4)	4 (13.3)	7 (12.3)	2 (4.5)	
Not known	2 (4.0)	0 (0)	0 (0)	1 (1.8)	0 (0)	
Anxiety preceding cystoscopy						0.972
Not anxious	27 (54.0)	14 (53.8)	17 (56.7)	32 (56.1)	26 (59.1)	
Some what anxious	17 (34.0)	8 (30.8)	10 (33.3)	19 (33.3)	15 (34.1)	
Very anxious	5 (10.0)	4 (15.4)	3 (10.0)	6 (10.5)	3 (6.8)	
Not known	1 (2.0)	0 (0)	0 (0)	0 (0)	0 (0)	
Median distance from clinic in miles (IQR)	6.3 (3.4-11.2)	9.9 (3.8-13.2)	6.1 (3.7-12.6)	5.5 (3.5- 11.8)	6.4 (3.3-11.5)	0.726

Table 8.5: Reasons for selecting cystoscopy or urinary test

A) Prefer cystoscopy	**N (%)**	**B) Prefer urinary test**	**N (%)**
I would feel anxious if I did not have a cystoscopy	90 (42.9)	I experience pain/ burning feeling when urinating after cystoscopy	64 (30.5)
I do not experience side effects after cystoscopy	82 (39.0)	I experience blood in urine after cystoscopy	49 (23.3)
Symptoms I experience after cystoscopy do not bother me	93 (44.3)	To avoid the risk of infection after cystoscopy	56 (26.6)
I have greater confidence in cystoscopy	148 (70.5)	I have greater confidence in the urine test	21 (10.0)
I prefer coming into the hospital for diagnostic tests	108 (51.4)	I experience no side effects after performing urinary test	59 (28.1)
The presence of a clinician makes me feel reassured	109 (51.9)	Having cystoscopy makes me feel anxious	30 (14.3)
The delay in receiving the results after urinary test bothers me	67 (31.9)	Urinary test does not require a hospital visit	60 (28.6)

8.3.1.1.4 Brief illness perception score

Overall, patients with NMIBC appear to be coping well following a diagnosis of cancer. Six months following a diagnosis of NMIBC, patients report that the disease has a minimal effect to their life, the symptoms they experience are minimal and that they are not particularly affected emotionally (Table 8.6). Patients are very optimistic that their treatment can control the disease and they acknowledge that they have a good understanding of the disease. However, they remain moderately concerned about bladder cancer. Most patients do not feel that they have personal control over their bladder cancer and believe that their illness will affect them for some time.

Correlation between patient demographics and clinical-pathological variables are shown in Table 8.7. Younger patients believed they had a better control of their illness and understand their illness better, compared to older patients. Patients with disease recurrence were more likely to believe their disease will be prolonged compared to patients with a new diagnosis. Higher grade, stage and disease risk were significantly associated with the effect of bladder cancer on the life of patients, degree of symptoms due to bladder cancer as well as the emotional effect of the illness. Patients' perception of disease recurrence risk affected them cognitively and emotionally more than the actual disease risk itself.

Table 8.6: Brief illness perception questionnaire scores

Component	median (IQR)
1) Consequence (n=205)	2 (0-5)
2) Timeline (n=193)	6 (3-10)
3) Personal control (n-202)	2 (2-5)
4) Treatment control (n-199)	9 (7-10)
5) Identity (n=202)	2 (0-5)
6) Concern (n-201)	5 (3-8)
7) Comprehensibility (n=204)	8 (6-10)
8) Emotion (n=202)	3 (1-6)
Overall score (n=184), median (IQR) (range)	32 (22-40) 0-65

Table 8.7: Patient demographics and clinical-pathological variables stratified according brief illness perception score.

Variables	Q1- consequence	Q2- timeline	Q3-personal control	Q4- treatment control	Q5- identity	Q6- concern	Q7- comprehensability	Q8- emotion
Age								
≤70 years	3.0 (2.5)	5.8 (3.5)	3.7 (3.1)*	8.1 (2.5)	2.9 (2.8)	6.0 (3.1)	8.2 (2.1)*	4.5 (3.1)*
>70 years	2.8 (2.6)	6.2 (3.5)	2.7 (3.5)	8.3 (2.5)	2.5 (2.7)	5.4 (3.3)	7.4 (2.9)	3.5 (3.0)
Gender								
Male	2.9 (2.6)	6.2 (3.5)	3.2 (3.3)*	8.3 (2.3)	2.6 (2.8)	5.4 (3.3)	7.6 (2.6)	3.5 (3.1)
Female	3.0 (2.6)	5.4 (3.7)	2.2 (2.5)	7.8 (3.1)	2.8 (2.7)	6.3 (3.1)	7.8 (2.8)	4.9 (3.1)
Highest education								
No formal education/ GCSE	3.0 (2.7)	6.5 (3.5)	2.7 (2.8)	8.2 (2.5)	2.8 (2.8)	5.8 (3.3)	7.6 (3.3)	4.1 (3.0)
A levels/degree holder	2.8 (2.5)	5.7 (3.4)	3.0 (3.0)	8.4 (2.1)	2.4 (2.6)	5.6 (3.2)	8.1 (2.0)	3.8 (2.3)
Previous cystoscopies, n (%)								
≤2	2.3 (2.2)	4.8 (3.5)	3.2 (3.3)*	8.0 (2.8)	2.0 (2.6)	5.1 (3.0)	7.6 (2.8)	3.4 (3.0)
2-5	3.3 (2.7)	5.9 (3.5)	3.3 (3.0)*	8.5 (2.1)	2.9 (2.8)	6.0 (3.1)	7.4 (2.6)	4.1 (3.2)
≥6	3.2 (2.8)	8.4 (2.3)**	1.9 (3.0)	8.0 (2.8)	3.1 (2.8)	5.5 (3.7)	8.2 (2.7)	3.8 (3.3)
New or recurrent cancer								
New	2.8 (2.5)	5.3 (3.5)	3.3 (3.1)	8.2 (2.4)	2.5 (2.7)	5.7 (3.1)	7.5 (2.6)	4.0 (3.0)
Recurrent	3.0 (2.7)	7.5 (3.1)*	2.4 (3.2)	8.2 (2.8)	2.7 (2.8)	5.4 (3.6)	8.0 (2.7)	3.4 (3.3)
Tumour grade								
G1	2.5 (2.3)	5.2 (3.6)	2.4 (2.9)	8.5 (2.2)	2.3 (2.6)	5.4 (3.2)	7.7 (2.9)	3.7 (3.2)
G2	2.6 (2.5)	6.1 (3.7)	3.3 (3.5)	8.3 (2.7)	2.4 (2.7)	5.3 (3.4)	7.8 (2.9)	3.3 (3.3)
G3	3.7 (2.7)*	6.3 (3.2)	3.2 (2.7)	7.9 (2.5)	3.4 (2.8)*	6.2 (3.0)	7.5 (2.2)	4.5 (4.5)
Tumour stage								
Isolated CIS/ pTa	2.6 (2.5)	5.7 (3.6)	3.3 (3.3)	8.4 (2.4)	2.4 (2.6)	5.3 (3.4)	7.8 (2.7)	3.4 (3.1)
pT1	4.2 (2.6)*	6.9 (3.1)*	2.4 (2.3)	7.6 (2.8)	3.9 (3.1)*	7.0 (2.4)*	7.1 (2.4)	5.1 (2.9)*
Actual disease risk								
Low	2.2 (2.2)	3.7 (3.4)	2.1 (2.9)	8.6 (1.9)	1.7 (2.5)	5.7 (3.2)	8.2 (2.6)	3.4 (3.0)
Intermediate	2.4 (2.5)	6.1 (3.7)	3.4 (3.5)	8.4 (2.6)	2.3 (2.6)	5.1 (3.4)	7.7 (3.0)	3.2 (3.0)
High	3.8 (2.6)**	6.4 (3.1)*	3.0 (2.7)	7.9 (2.5)	3.4 (2.8)**	6.3 (2.9)	7.5 (2.2)	4.6 (3.1)*
Patient perception of risk of recurrence								
Low	1.9 (2.3)	4.5 (4.0)	3.7 (3.8)	7.8 (3.3)	1.6 (2.6)	3.6 (3.2)	7.5 (3.3)	2.2 (2.7)
Intermediate	2.8 (2.4)	6.2 (3.2)	2.9 (2.9)	8.3 (2.1)	2.8 (2.7)	6.1 (2.8)	7.5 (2.5)	4.2 (3.0)
High	4.5 (3.0)**	8.2 (2.8)**	2.5 (2.9)	8.5 (2.7)	3.6 (3.0)**	6.9 (3.5)**	8.8 (1.8)*	4.7 (3.6)**
Any adverse event								
Yes	3.0 (2.7)	6.3 (3.4)	3.2 (3.2)	8.4 (2.4)	2.9 (2.8)*	5.7 (3.3)	7.9 (2.6)	3.8 (3.2)

No	2.4 (2.2)	5.1 (3.7)	2.5 (3.0)	7.6 (2.8)	1.8 (2.5)	5.3 (3.0)	7.1 (3.0)	3.6 (2.8)
Haematuria								
Yes	3.4 (2.7)*	6.9 (3.2)*	3.3 (3.2)	8.5 (2.3)	3.1 (2.8)	5.9 (3.2)	8.0 (2.2)	4.0 (3.2)
No	2.5 (2.4)	5.7 (3.6)	2.5 (3.1)	7.9 (2.6)	2.3 (2.7)	5.3 (3.2)	7.4 (3.0)	3.7 (2.9)
Dysuria/ LUTS								
Yes	3.0 (2.6)	6.5 (3.4)*	3.2 (3.1)	8.3 (2.4)	2.9 (2.7)	5.7 (3.3)	7.8 (2.5)	3.9 (3.2)
No	2.6 (2.7)	5.2 (3.7)	2.6 (3.2)	8.0 (2.8)	2.1 (2.8)	5.2 (3.1)	7.4 (2.9)	3.6 (3.0)
UTI requiring antibiotics								
Yes	3.6 (2.9)	6.5 (3.3)	3.2 (3.4)	8.2 (3.0)	3.6 (3.1)*	5.9 (3.4)	8.0 (2.5)	4.3 (3.5)
No	2.7 (2.5)	6.1 (3.6)	2.9 (3.1)	8.3 (2.3)	2.4 (2.6)	5.4 (3.2)	7.6 (2.7)	3.6 (3.0)

* denotes $p<0.05$, ** denotes $p<0.01$

8.3.1.2 Qualitative analysis

8.3.1.2.1 Patient demographics

The demographics and tumour characteristics of the 20 patients interviewed are shown in Table 8.8. Median patient age was 73.7 years which was similar to the full 213 patient cohort. Twenty percent of patients were of low risk disease to ensure adequate representation of low risk NMIBC, which has minimal risk of disease progression.

Table 8.8: Patient demographics and tumour characteristics of patients in the qualitative sub-study

Variable	**N (%)**
Age, median (IQR)	73.7 (61.8, 80.2)
Gender, n (%) **Male**	 15 (75)
Tumour grade, n (%) **G1** **G2** **G3**	 4 (20) 8 (40) 8 (40)
Tumour stage, n (%) **pTa** **pT1**	 15 (75) 5 (25)
Disease risk, n (%) **Low** **Intermediate** **high**	 4 (20) 8 (40) 8 (40)
Recent recurrence ≤6 months, n (%) **Yes**	 5 (25)

8.3.1.2.2 Emerging qualitative themes

The main themes that emerged were classified to 1) Views and experience of cystoscopy, 2) Views and experience of urine test and 3) Active comparison between cystoscopy and urine test.

8.3.1.2.2.1 Views and experience of cystoscopy

Patients appreciate the fact that cystoscopy provides a visual diagnosis of cancer and attributed this to a presumed near perfect sensitivity for the detection of bladder cancer. While patients do not like cystoscopy, they were prepared to tolerate it due to its good diagnostic ability. Patients also value the fact that cystoscopy provides an instant diagnosis and appreciate that a healthcare professional is performing cystoscopy.

Patient perception of passing a cystoscope along the urethra can be disturbing although they recognise the requirement to visualise the bladder. Some patients described the procedure as embarrassing and felt violated following cystoscopy. Patients also appreciate that cystoscopy performed by an experienced urologist would reduce adverse events and patient discomfort. Qualitative analysis is shown in Table 8.9.

Table 8.9: Qualitative analysis for advantages and disadvantages of cystoscopy- excerpt from patient interviews.

Advantages of cystoscopy
Visual diagnosis "It's [bladder cancer] caught on camera as it were. You can literally see what's going on" "I know it's there and it's [bladder cancer] staring right at me. You literally see and discuss what's there to the chap or women who is doing it" "The fact that the camera shows you that thing on the camera and that they show a scar and they do a grand tour of my bladder. It's reassuring to see that"
Confidence in diagnostic accuracy "I think it's quite accurate. I would say 95%. It found mine and mine was really tiny, a couple of mm. They picked it up with the camera and there it was sitting on the wall. It looked like a little sea emomey"

“They actually have a camera on the end and its magnified they can see anywhere in the bladder. So, to me, that’s accurate. If it wasn’t, they wouldn’t have found mine” “I presume it’s because it’s on the screen. I mean I’m not a doctor. I can only assume that what you see is what you get sort of thing. I mean, there it is.... it’s a cancer. And there is this on the screen I suppose that must be 100% identifiable” “To be honest, I think it [cystoscopy] is the only way we can know for sure is there anything there or not”
Tolerability of cystoscopy “Cystoscopy is something I got used to. I have had quite a few of those now and I accept that fully” “Well I don’t like them but I want to have the most accurate diagnosis possible” “If it’s the only way then it’s best to know what’s going on but it’s not exactly a great overall experience. So, if there’s an alternative obviously, it’s preferable” “It is quite invasive but I think I preferred that because I think then you know that you got an accurate reading of what’s going on” “It’s embarrassing obviously because the thought of exposing yourself to people but it’s necessarily at the same time. So, it’s overcoming one thing or the other” “Well I don’t suppose there is any other way to do it” “You know I really have no dignity left for the start. But you know it’s a small price to pay” “I mean even though a bit of uncomfortable, I don’t mind having the camera probe”
Instant diagnosis “I can literally walk out of there knowing that all is well and that’s very helpful” “..that’s [cystoscopy] quite a reassurance to walk out of there thinking that, that month was all right and we go on from here” “…. it was a bit daunting but it was instantaneous. There wasn’t any waiting around” “I can see all in front of me. I guess when someone does a blood test you’ve got to wait two three weeks for the results to come back”
Qualified person “But if you have a qualified person who takes a look inside your bladder with a camera. That’s as good as any I think”

"They do it at the very professional way and it's a reassurance for me" "…the person who did it said it was all clear, so you know... nothing else to go off really"
Disadvantages of cystoscopy
Invasive Well the thought of a camera going inside me from the place they put it in my urethra….the thought of that going into me does put me off. I don't really like that but you know where else can it go…. I feel that the best entry point…. not being cut you open" "It was just, obviously the fact that my tube had been invaded with an alien peace… It was just that I have never gone through anything like this before and it was not what I was expecting…"
Adverse events "The urine was burning at first but that goes away after a couple of hours… So, what I do is I drink plenty of water and just flush it all through" "….I did have a bladder problem where I couldn't control the use of my bladder whereas when I had a feeling there…. I had to pee you know straight away… Rushing to the toilet is most cases" "It was the after effects that went wrong. I couldn't pass no water but that's sorted itself out now" "Well it must have been sort of the first couple of passes, a little bit of blood came out after that, it was okay"
Embarrassing "It's embarrassing obviously because the thought expose yourself to people but it's necessarily at the same time. So, it's overcoming one thing or the other" "Again, it's a psychological embarrassing feeling that well okay I'm exposing myself to somebody.." "You know I really have no dignity left for the start"
Operator dependent "…the doctor was being shown how to use a new machine. Two other people watching and a lady showing her what to do you know. It didn't bother me at the time but you know I was a bit sore. I was well quite sore after that. It took me a week or so to get myself right with it. Now the second, the next time I went for it, it was the registrar… I couldn't believe it. He did it and he said right okay thank you.

So, I said, “are we done” and he said, “yeah”. With the other lady, .it seemed like an hour but this was probably about 10 minutes. Well it was only minutes with this chap. I just walked out as good as when I went in”

8.3.1.2.2.2 Views and experience of urine test

Patients value the convenience of a urine biomarker, reducing the need to attend hospital and be subjected to a procedure. Furthermore, patients appreciate that a urine biomarker is free of adverse events unlike cystoscopy and believe that a urine biomarker will allow for earlier testing and subsequently allow for prompt commencement of treatment. Full qualitative analysis is shown in Table 8.10.

Table 8.10: Qualitative analysis for advantages of urine test- excerpt from patient interviews.

Advantages of urine test
No adverse events “If they can find it all the way through urine test… that would be a much better and more comfortable way of checking” “..certainly more convenient and less uncomfortable for the patient”
Less intrusive “…if it helps detect cancer in a less invasive way I suppose it is good” “.. there’s less interference in there, ... I’ve always been a bit sore when I’ve had one [cystoscopy]. You know you go for a wee and it sore” “… the urine test is much less alarming thing to do than going in for a cystoscopy” “… reduce the level of personal invasions”
Quicker treatment time “…where you could start treatment early I think would be a great advantage”
Reduce patient embarrassment

“It’s not a very nice experience you know…there were two young girls in their 20s. Nurses. It’s not a nice experience anyway but having that it’s a bit.... you know, not nice”
Convenience “I suppose logically the urine test if it’s proven is a bit easier” “… it would be a simple thing to collect some urine and see you could determine whether there were cancer cells, where you could start treatment early I think would be a great advantage” “Obviously a lot easier than the cystoscopy” “…reduce inconveniencing the patient” “…if I could find out everything from urine sample then it would be a lot easier because you don’t have to spend any time in the hospital” “..certainly more convenient and less uncomfortable for the patient”

8.3.1.2.2.3 Active comparison between cystoscopy and urine test

When comparing between cystoscopy and the urine biomarker, patients are pragmatic and understand that no test is 100% accurate. Patients prioritise the test with the highest sensitivity and most would only accept a urine test with a similar sensitivity to cystoscopy. Missing bladder cancer during surveillance is a significant worry to patients and patients with high grade bladder cancer felt particularly concerned about missing recurrence and prioritise the high sensitivity of cystoscopy. Some patients’ familiarity with cystoscopy and the fact that they had a positive experience with cystoscopic detection of cancer reinforced their preference for cystoscopy over a urine test. An overarching theme was that patients were not confident in the ability of a urine test to identify bladder cancer with a high sensitivity as they perceived it as ‘experimental’ when compared to cystoscopy, the current gold standard.

Patients who experienced a previous embarrassing experience related to cystoscopy were willing to accept a lower diagnostic sensitivity for a biomarker. All patients were open to interspacing cystoscopy with a urine biomarker to increase the interval between cystoscopies, although most reinforced the requirement for comparable sensitivity. Some patients expressed the opinion that a molecular urine test may potentially identify cancers before they are diagnosed visually. Further, some patients were sceptical about the ability of a biomarker which will be able to match cystoscopy. Qualitative analysis is shown in Table 8.11.

Table 8.11: Qualitative analysis for active comparison between cystoscopy and urine test and patient scepticism about urine test- excerpt from patient interviews.

Active comparison between cystoscopy and urine biomarker
"Given the particular cancer I have is high grade…signet cell variation…. I'd be wary of it [urine biomarker]. I need more reassurance as I am not out of the woods yet Because I'm just a year into a disease. Everything is happening well for me at present the treatment seems to be working well for me and I'm very relaxed and confident about it. I would need some reassurance that this is as good or comparable"
"Well I am not fussed either way…that [urine biomarker] would be an easier way obviously instead of going through cystoscopy but I don't know how accurate it is going to be…If you do pull it off then all well and good"
"I'll look at it differently. You know I'm 79, a realist… I am contempt, happy with the treatment. And the cystoscopy is something that has become part of my life and I'm content with that"
"Well I still would like it [urine biomarker] to be up to 99 percent. It has to be. You can't mess with peoples' life. You can't have 70-78% and then it's quite possible you missed it. You know. if its 99-99.5 percent, at least you are in the right area with cystoscopy"
"I think it must be comparable to the cystoscopy. Otherwise, the numbers that could slip through would be unfortunate"
"Well there is no 100% guarantee here, but a high percentage would be good"

"Even if the percentages weren't as good, I would prefer to have the urine test. It's [cystoscopy] not a very nice experience you know. The last time I went, there's two young girls in their 20s. Nurses. It's not a nice experience anyway but having that is not nice"

"Like I said, I don't really believe in…Well I mean if you can positively detect cancer in that fashion then it will be so good. But I like I like physical checks as well so like I said that the cystoscopy often a good idea too"

"Cystoscopy, you could only see a visual… the urine test could detect earlier then the visual one…. you could only see as far as the eye can see"

"I don't know. I am not a doctor. I would follow the advice of the doctor, wouldn't you?"

"I'm happy with this is cystoscopy because I know it's working"

"I would always prefer whichever is most accurate"

"I want as much of certainty as possible"

"I would just like to know definitely rather than not so sure"

Patient scepticism about urine test

"I would need some reassurance that this is as good or comparable"

"You will need to reassure me with the evidence. I suppose logically the urine test if it's proven which is a bit easier. But I'm not moved yet to trust it. Not without some evidence"

"Yeah, the percentages don't weight up at the moment, really. You know, if it's only a 40 percent chance of success, I would stick to the other I would? So, I was more uncomfortable but if we put up with it you will be sure it will be alright"

"I worried that the water sample, whether that would be as good as the cystoscopy"

"I'm sure it [use of urine biomarker] will happen. Maybe not yet. I'm happy with this is cystoscopy because I know it's working"

"What is the aim? I imagine it is to cut down the number of flexible cystoscopy which is very expensive in terms of hospital time and staff time and so forth. Is that the basis of it?"

"I think it [urine biomarker] will still be playing at the back of my mind whether it was accurate or not"

“Also, the severity... I had a very mild one, a very small growth. As someone with a more aggressive and bigger…then it might be better with the to have a camera”

8.4 Discussion

This represents the first study to qualitatively explore the views and decision making of patients when considering between cystoscopy and a urine biomarker for the detection of bladder cancer recurrence. Majority of patients (75.8%) recognise biomarker performance as important and would discount any biomarker with MAS <90%. However, 63.3% of patients would accept a biomarker with a MAS of ≥95% suggesting that patients' willingness to accept a biomarker is linked to its performance characteristics. Nevertheless, 21.3% of patients would prefer cystoscopy regardless of urine biomarker performance because of factors such as immediate readout and clinician interaction despite having to travel to a hospital.

Patient acceptance of cystoscopy was independent of experience of adverse events relating to the test suggesting that the high sensitivity of cystoscopy of paramount importance. This is similar to data from colonoscopy where patients consider a high sensitivity to be of paramount importance with risk of adverse effects a secondary concern (351). Our data suggest that the prevalence of complications following cystoscopy are not negligible with 50% of patients' self-report haematuria and urinary symptoms following cystoscopy and 24% developing UTI requiring antibiotics. This is considerably higher compared to previous reports although this represents a cumulative experience of patients who have had multiple cystoscopies and not an incident rate (20).

We did not observe an association between patient demographics, education level and clinical-pathological variables with a lower MAS. In addition, higher disease stage, grade, actual risk or patient perceived risk classification was not

associated with a higher acceptable sensitivity. The data indicate that even patients with a lower risk of recurrence or progression place a high emphasis on accurate cancer detection. Patients have a perceived benefit for early detection of recurrence which is clear in high risk bladder cancer although limited data exist to support this in low risk cancers. Observational reports suggest that active surveillance of G1 pTa NMIBC patients does not increase oncological risk (352). Distance to hospital was not a factor, as patients value the visit to hospital and prefer seeing a clinician as it reassures them.

Two previous studies reported the MAS required of a urine biomarker to replace cystoscopy in NMIBC patients in the surveillance setting. Vriesema and colleagues surveyed 102 patients with at least 12 months follow-up and reported that 89% of patients will not accept a urinary biomarker with a sensitivity of <90% (353). Yossepowitch and colleagues assessed the preference of 200 patients having check cystoscopies at various time points and reported that 75% of patients will not accept a biomarker with an accuracy of <95% (318). Both studies reported that male patients were willing to accept a marginally lower MAS (318, 353). The study by Vriesema et al. and Yossepowitch et al. report that patients who were older (>67 years) and those who experienced a higher pain intensity following cystoscopy respectively were significantly more likely to accept a lower sensitivity (318, 353).

The current study differs from these two studies. Besides reporting the MAS of a urine biomarker patients were prepared to accept, we also interrogated reasons for patients' preference for cystoscopy of urine biomarker using qualitative and quantitative methods. We did not find any variables associated with a lower MAS. A reason for this may be the fact that patients in the current study completed the

questionnaire at 6 months following a cancer diagnosis suggesting shorter time interval compare to the other two studies. The fear of cancer recurrence may be prioritised over pain attributed to cystoscopy (318, 353). Patients in both studies also did not experience the use of a urine biomarker and assumed that cystoscopy was 100% sensitive which is not in clinical practice.

Patients value the visual element of cystoscopy as this is something they can relate to and the absence of a visual diagnosis of cancer provides significant reassurance. Patients are aware that cystoscopy is the gold standard diagnostic test for bladder cancer surveillance and perceived a urine test as experimental which may introduce bias. However, if the sensitivity of a urine test is proven to be close to cystoscopy, 78.7% of patients would be happy to accept a urine test at intervals.

For the first time, we reported the cognitive and emotional state of NMIBC patient using the validated BIPQ. Overall, patients with NMIBC reported a better cognitive and emotional state compared to endometrial, colorectal, non-Hodgkin's lymphoma and myeloma (354). There patients were less affected by their cancer, are more optimistic about their treatment, report less symptoms and have a better understanding of their illness. However, patients with NMIBC report that their disease is more likely to continue, which may be due to the fact that all patients were assessed 'soon' after a diagnosis of bladder cancer (6 months) and that some patients with bladder cancer will require life-long cystoscopy surveillance. Similar to other reports, patients with more adverse oncological features were more likely to be concerned about their cancer, this affected them emotionally and in their overall life (354).

8.5 Limitations

Limitations to the current study should be acknowledged. Complications such as UTI following cystoscopy was self-reported by patients and not confirmed by urine culture. It was assumed that patients would comprehend the questionnaire when completing it as no medical terminology was used. Although all patients provided a urine sample for biomarker testing, they were not provided the results of the biomarker. The knowledge of the biomarker results combined with a patients' experience with cystoscopy may affect the MAS.

8.6 Conclusion

I report that patients with NMIBC value the high sensitivity of cystoscopy despite patient discomfort and adverse events following cystoscopy. The lack of association between patient demographics and adverse cancer features with MAS suggest that all patients consider any cancer significant and are not willing to compromise on the diagnostic ability of a test. Hence, a diagnostic sensitivity of any urinary biomarker must be close to that of cystoscopy before patients are prepared to accept it over cystoscopy.

CHAPTER 9 : OVERALL CONCLUSION

The results in this thesis suggest that existing guidelines used to determine which patients should undergo investigations following a presentation of haematuria have significant limitations. The use of arbitrary age thresholds and type of haematuria alone such as the ≥45 years for VH and ≥60 years for NVH to recommend investigations for patients presenting with haematuria as recommended by NICE will miss a significant number of urinary tract cancers. In contrast, utilising age thresholds recommended by the AUA of ≥35 years for NVH patients and all VH patients will identify more urinary tract cancers as well as increasing the number needed to screen. My results showed that incorporating a risk assessment approach incorporating additional variables such as smoking history and gender improves case selection of patients with haematuria who should have investigations.

There are several caveats to the results reported. The DETECT I study recruited patients referred for haematuria investigations at secondary care hence the reported incidence of urinary tract cancer of 9.9% (13.5% for VH, 3.1% for NVH) may not be applicable to patients seen in primary care. This is due to case selection bias where primary care physicians may only refer patients with haematuria who they deem as high risk such as patients who are older and those with a smoking history. Analysis of primary care records suggest that that VH has a PPV of 2.8% and NVH a PPV of 1.6% in patients ≥60 years although this is an underestimate given the fact that haematuria due to UTI may be included in this patient cohort (150). The true incidence of urinary tract cancer is likely to be somewhere between the incidence I have reported in this thesis and the incidence reported from the study which utilised primary care records (150).

It is important to point out that any guidelines or recommendations for haematuria should be tasked to maximise cancer detection while minimising the number of patients needed to screen. Hence, the aim should not be to identify all urinary tract cancers but to ensure majority of cancers, particularly high risk disease, are identified. Patients with haematuria with a low risk of cancer can be monitored for recurrent symptoms which can inform the decision to investigate. The added complexity is the fact that although the incidence of urinary tract cancer in NVH is low, not investigating this patient cohort can have repercussions due to the fact that 57.6% and 30.3% of NVH patients with a diagnosis of bladder cancer have high risk and MIBC disease respectively.

Screening asymptomatic patients without symptoms is not recommended due to the low incidence of urinary tract cancer in the general population. Hence, haematuria is often used as a 'red flag' sign to trigger investigations. A screening guideline attempting to identify all urinary tract cancers will be costly and will subject a significant number of patients to invasive investigations such as cystoscopy and radiation in the form of CT imaging as well as the unnecessary anxiety presiding test results.

Incorporating a nomogram approach as my results suggest will allow better patient selection where more urinary tract cancers will be identified compared to NICE guidelines with a more favourable number needed to screen compared to the AUA guidelines. The required threshold can be calibrated for sensitivity for cancer detection and this may help select patients who have a higher risk of urinary tract cancer to have expedited haematuria investigations to prioritise prompt cancer diagnosis to allow for earlier treatment. The HCRS I developed utilises clinical variables which are already collected as part of a standard

assessment of patients with haematuria. It is early to use and can be incorporated in a busy clinic. While it has been validated in an external patient cohort in the secondary care setting, it remains unvalidated in primary care and this will be required prior to adoption. Further, acceptance by primary care physicians will need to be accessed although I anticipate that this will not be an issue if the HCRS has a high discriminatory ability to reduce the number of patients subjected to haematuria investigations.

I also evaluated what investigations should be use when evaluating patients with haematuria. My results confirm that the sensitivity of urine cytology, CTU and RBUS, in isolation of in combination, is insufficient to replace cystoscopy. Hence, cystoscopy remains the gold standard to detect bladder cancer. However, I reported that RBUS can safely replace CT urogram for upper tract imaging in patients presenting with NVH. The low incidence of UTUC in NVH patients and the high sensitivity of RBUS for the detection of RCC suggest that this is safe and the risk of failing to detect UTUC is remote. However, I acknowledge that not all patients with NVH had both RBUS and CT urogram. Further, patients with a normal RBUS would have been discharged without a CT urogram hence, it is possible that some of these patients had a false negative test.

With regards to the role of urine cytology as an adjunct to cystoscopy and upper tract imaging, my results suggest that urine cytology adds little value and would subject patients to further unnecessary investigations due to a high risk of false positives. Even in high grade disease, the sensitivity of urine cytology remains low. While the use of routine urine cytology in the haematuria setting has been abandoned in most centres, it is still used in 22.5% of hospitals which recruited patients to DETECT I and this should be discouraged.

There is a clear need for a non-invasive urine biomarker test both in the haematuria as well as the NMIBC surveillance setting. Current commercially available urine biomarkers have a sensitivity of <80% which is insufficient to replace cystoscopy. While numerous novel biomarker panels have been reported, they remain unvalidated in a prospective clinical trial setting. As reported in my systematic review, multi-target panels have a better diagnostic performance compared to single target biomarkers. Further, there may be an advantage in combining more than one 'omic' class biomarkers to improve assay sensitivity.

The development of the UroMark and subsequent small scale external validation showed promise with a reported AUC of 97% and corresponding sensitivity of 98% and specificity of 97% (140). The DETECT I study was to validate the UroMark in a prospective observational study of haematuria patients with a pre-planned power calculation. While analysis of the full patient cohort is currently ongoing, the interim analysis of the UroMark confirmed a sensitivity of above 90%. Pre-planned power calculations required a total of 890 urine samples from haematuria patients, with an enrichment cohort of a further 380 urine samples from bladder cancer patients (1,270 in total). A total of 3,556 patients were recruited to DETECT I, of which 2,676 patients (75.3%) provided a urine sample. This represents a large biorepository which will be useful for future biomarker validation studies due to the excess DNA extracted from these urine samples.

While this study is amongst the first urine biomarker prospective validation study with a pre-planned power calculation, several methodological limitations exist. Urine samples were collected following cystoscopy and not in hospital prior to cystoscopy. This was because in our early experience, urine samples collected

in clinic prior to cystoscopy were of low volume and contact time of urine with bladder urothelium was short resulting in a low DNA yield which was insufficient for the assay. Urine samples were not collected prior to hospital attendance for haematuria investigations due to issues with patient consent. It was decided that urine samples were collected following hospital visit at home which will result in a high DNA yield due to a prolonged urine contact time with bladder urothelium. Patients were advised to collect urine samples at least 48 hours following cystoscopy to reduce the risk of urothelium cell shedding following cystoscopy and cystodistension. Our early experience suggests that home urine collection before and after cystoscopy had a similar DNA yield.

I also acknowledge that the study design required enriching the prospective haematuria cohort of 890 patients with urine samples from a further 380 bladder cancer patients. This allows for a more achievable patient recruitment target although I acknowledge that ideally recruiting consecutive haematuria patients without enrichment would be advantageous. Without enriching the patient cohort, I would need to recruit 5,429 consecutive patients with haematuria to collect 3,800 urine samples (based on a 70% response rate), which would be costly and resource demanding.

Finally, using a mixed method approach of combining patient questionnaires and qualitative assessment by semi-structured interviews, I report that although patients can experience adverse events following cystoscopy, they would still only accept a urine-based test with a sensitivity comparable to cystoscopy. Patients with both low and high-risk disease were not prepared to compromise on a high diagnostic sensitivity for the convenience and comfort of a non-invasive test. Further, I report that patients with NMIBC had a better cognitive and

emotional state compared to endometrial, colorectal, non-Hodgkin's lymphoma and myeloma based on the brief illness perception score, which has not been reported in the NMIBC setting (354).

There is no doubt that with the advent of NGS, advances in genomics and declining cost of sequencing, urine-based biomarkers for the detection of cancer will be a reality in the coming decade. I anticipate, that incorporating both clinical variables as part of a risk assessment approach together with genomic test in a form of a urinary biomarker will better select patients requiring investigations following a presentation of haematuria, which may ultimately reduce the requirement for cystoscopy. Such an approach will potentially revolutionise haematuria investigation and bladder cancer surveillance pathways and have a profound impact on the requirement for cystoscopy, patient well-being as well as reduction in healthcare costs.

CHAPTER 10 : FUTURE WORK

The cumulative work of this thesis represents contemporary incidence of urinary tract cancers in patients referred for haematuria to secondary care as well as an assessment of the diagnostic performance of investigations used in the evaluation of haematuria. This subsequently led to the development of the HCRS which has been validated in secondary care. I report the interim analysis of the diagnostic performance of the UroMark. Further analysis of the urinary DNA from patients recruited into DETECT I and II studies are ongoing and will be reported separately to this body of work.

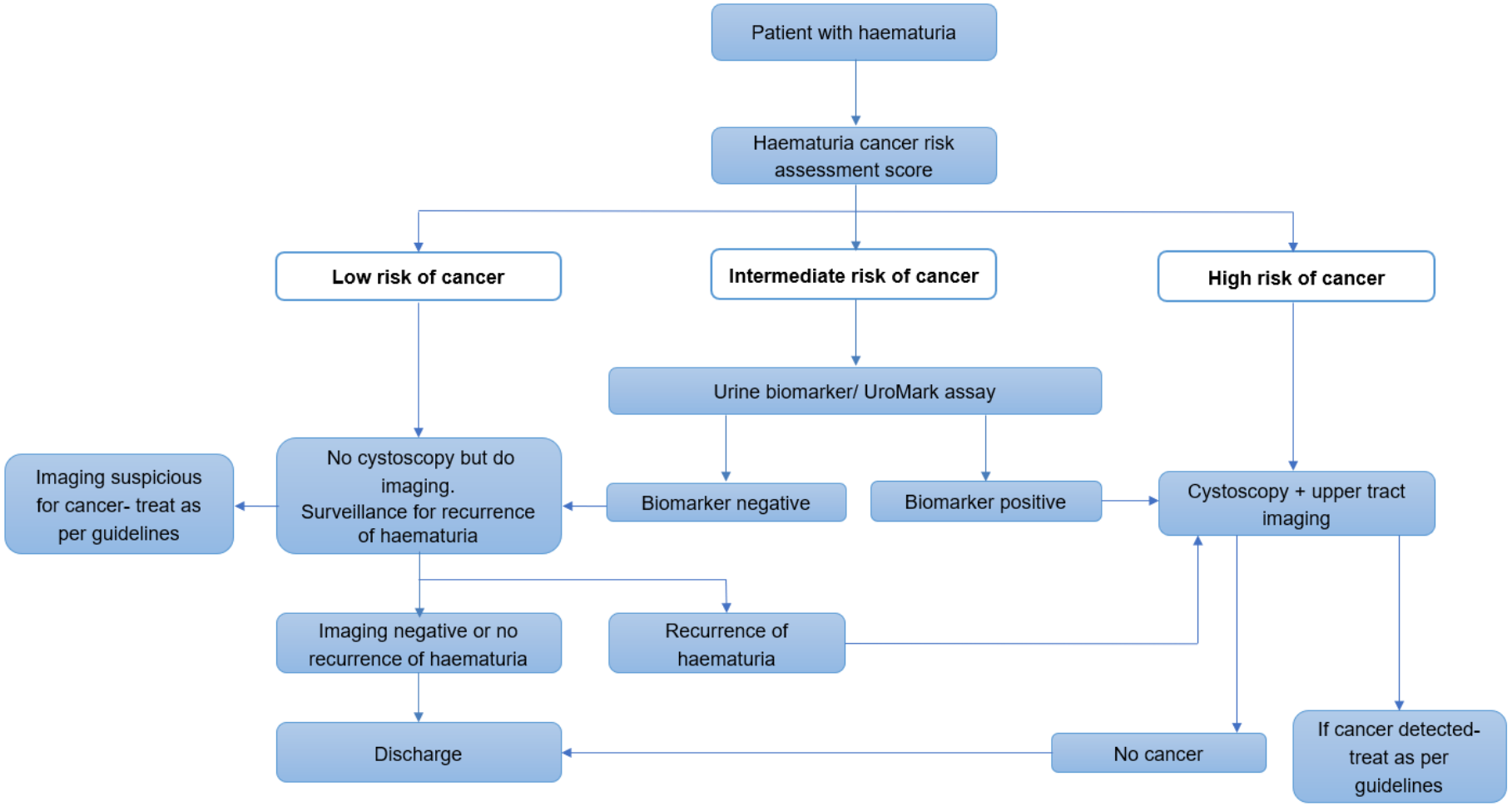
I have made contact with Mr Fadel Mishriki (Consultant Urologist, Aberdeen Royal Infirmary), who has a prospective annotated series of over 2000 UK patients with haematuria which I intend to use as a UK validation cohort to test the HCRS. I subsequently hope to test the HCRS in a primary care patient cohort which would reduce case selection bias where not all patients with haematuria in the absence of UTI may be referred to evaluation in secondary care. The Kelly-Feber research group has links with Prof Willie Hamilton (Professor of Primary Care Diagnostics, University of Exeter), who has previously published using primary care patient records in patients with haematuria.

Subsequent work would involve a decision tree analysis and a cost effectiveness analysis of diagnostic test used as part of haematuria investigations. I spent 8 weeks at the Brigham and Women's Hospital, Harvard Medical School, Boston, USA in the summer of 2018 and have been working with Prof Steven Chang (Assistant Professor in Surgery, Brigham and Women's Hospital, Harvard Medical School) who has expertise in Markov modelling, cost effectiveness and decision analysis.

Once the urinary DNA from the DETECT I patient cohort have been assayed using the UroMark platform, I intend to test the utility of epigenetic panel in combination with a risk assessment score to determine if patient selection for haematuria can be optimised and the use of cystoscopy can be minimised. This would lead to the development of a haematuria algorithm where patients with the highest risk based on a risk assessment score would have cystoscopy with upper tract imaging and those with the lowest risk of urinary tract cancer would avoid cystoscopy but actively surveyed for the representation of haematuria. Patients with an intermediate risk score would have a UroMark assay or any other high performance urine biomarker test, which would then guide the requirement for cystoscopy should they fall into the high risk category (Figure 10.1). This algorithm can then be tested in a randomised prospective setting where the algorithm would be compared to current guidelines recommendations. The successful recruitment in patients into DETECT I and II suggest such a trial is feasible and will provide level one evidence to support a novel haematuria diagnostic pathway.

The subsequent phase in the UroMark programme is to prospectively test the assay in the NMIBC surveillance setting. I was a co-lead applicant of a Cancer Research UK Biomarker Discovery Grant to test the UroMark assay in the NMIBC surveillance setting. This application was unsuccessful and I intend to reapply for further funding following the publication of the results validating the UroMark in the haematuria setting (DETECT I study).

Figure 10.1: Proposed haematuria algorithm incorporating the haematuria cancer risk score and urine biomarker/ UroMark assay



CHAPTER 11 : REFERENCE & APPENDIX

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11.2 Appendix

A1: Centres recruited patients for DETECT I

Hospital	Site opened	Patients
Darent Valley Hospital	09.08.16	122
Derriford Hospital	03.01.17	125
Doncaster Royal Infirmary	19.10.16	75
Dorset County Hospital	24.11.16	92
East Lancashire Hospitals NHS Trust	24.11.16	130
East Surrey Hospital	12.05.16	261
East Sussex Healthcare NHS Trust	05.10.16	62
Homerton Hospital	27.07.16	101
James Cook University Hospital	20.06.16	161
Kent & Canterbury Hospital	12.10.16	58
Kettering General Hospital	06.01.17	48
King's Mill Hospital	06.01.17	30
Macclesfield Hospital	23.09.16	96
Maidstone Hospital	06.10.16	211
Medway Maritime Hospital	23.09.16	77
New Cross Hospital	07.11.16	60
Norfolk & Norwich University Hospital	19.08.16	75
North Devon District Hospital	23.09.16	120
Northern Lincolnshire & Goole NHS Trust	23.09.16	70
Northwick Park Hospital	02.08.16	75
Pennine Acute Hospitals NHS Trust	09.11.16	147
Pinderfields Hospital	12.01.17	15
Royal Albert Edward Infirmary	18.01.17	47
Royal Bolton Hospital	29.11.16	87
Royal Cornwall Hospital	27.01.17	65
Royal Devon and Exeter Hospital	23.09.16	56
Royal Surrey County Hospital	16.08.16	77
Salford Royal Hospital	27.10.16	102
Salisbury District Hospital	05.10.16	110
St James’s University Hospital	14.10.16	18
Stepping Hill Hospital	23.12.16	72
Tameside General Hospital	21.11.16	116
University College London Hospital	23.03.16	196
University Hospital of North Tees	29.09.16	66
University Hospitals Coventry	12.01.17	8
Western Sussex Hospitals NHS Trust	13.12.16	262

A2: Centres recruited patients for DETECT II (as of April 2018)

Hospital	Site opened	Patients
Barking, Havering and Redbridge University Hospitals NHS Trust	06.03.17	9
Broomfield Hospital	14.06.17	35
Burton Hospitals NHS Foundation Trust	21.04.17	11
Charing Cross Hospital	20.03.17	6
Darent Valley Hospital	09.06.17	33
Derriford Hospital	22.08.17	11
Dorset County Hospital	24.04.17	29
East Lancashire NHS Trust	24.11.16	20
East Surrey Hospital	03.11.16	51
East Sussex Healthcare NHS Trust	27.06.17	9
Guy's & St Thomas Hospital	01.06.17	33
Hillingdon Hospital	05.06.17	31
Homerton Hospital	25.04.17	2
James Cook University Hospital	19.09.17	7
Kent & Canterbury Hospital	04.04.17	9
Kettering General Hospital	23.05.17	22
King's Mill Hospital	06.01.17	71
Leicester Royal Infirmary	27.06.17	24
Macclesfield Hospital	14.02.17	13
Maidstone Hospital	09.03.17	39
Manchester University Foundation Trust	04.04.17	37
Medway Maritime Hospital	14.11.16	36
New Cross Hospital	05.12.16	22
Norfolk & Norwich University Hospital	27.10.16	31
North Devon District Hospital	05.05.17	24
North Middlesex Hospital	07.03.17	33
Northern Lincolnshire & Goole NHS FT	25.10.16	45
Royal Albert Edward Infirmary	18.01.17	27
Royal Bolton Hospital	29.11.16	9
Royal Cornwall Hospital	17.03.17	22
Royal Derby Hospital	19.06.17	10
Royal Free London NHS Foundation Trust	21.02.17	18
Royal Lancaster Infirmary	30.08.17	16
Royal Preston Hospital	04.10.17	18
Royal Shrewsbury Hospital	15.02.17	30
Royal Surrey County Hospital	02.08.17	2
Salisbury District Hospital	03.03.17	14
Southend University Hospital NHS FT	14.06.17	19
St James's University Hospital (Leeds)	09.05.17	14
Stepping Hill Hospital	23.12.16	41
Tameside General Hospital	21.11.16	37

The Pennine Acute Hospitals NHS Trust	18.01.17	43
University College London Hospitals	15.09.16	34
University Hospital Coventry	12.01.17	27
University Hospital of North Tees	31.01.17	0
West Middlesex University Hospital	01.08.17	19
Western Sussex Hospitals NHS Foundation Trust	13.12.16	75
Yeovil District Hospital	28.03.17	34

A3: PROSPERO registration for systematic review

PROSPERO

Systematic review

1. * Review title.
Give the working title of the review, for example the one used for obtaining funding. Ideally the title should state succinctly the interventions or exposures being reviewed and the associated health or social problems. Where appropriate, the title should use the PI(E)COS structure to contain information on the Participants, Intervention (or Exposure) and Comparison groups, the Outcomes to be measured and Study designs to be included.
Novel urinary biomarkers for the diagnosis of bladder cancer: a systematic review 2. Original language title.
For reviews in languages other than English, this field should be used to enter the title in the language of the review. This will be displayed together with the English language title.
3. * Anticipated or actual start date.
Give the date when the systematic review commenced, or is expected to commence.
01/06/2016
4. * Anticipated completion date.
Give the date by which the review is expected to be completed.
01/11/2017
5. * Stage of review at time of this submission.
Indicate the stage of progress of the review by ticking the relevant Started and Completed boxes. Additional information may be added in the free text box provided.
Please note: Reviews that have progressed beyond the point of completing data extraction at the time of initial registration are not eligible for inclusion in PROSPERO. Should evidence of incorrect status and/or completion date being supplied at the time of submission come to light, the content of the PROSPERO record will be removed leaving only the title and named contact details and a statement that inaccuracies in the stage of the review date had been identified.
This field should be updated when any amendments are made to a published record and on completion and publication of the review.
The review has not yet started: No
Review stage
Started
Completed
Preliminary searches
Yes
Yes
Piloting of the study selection process
Yes
Yes
Formal screening of search results against eligibility criteria Yes
Yes
Data extraction
Yes
Yes
Risk of bias (quality) assessment
Yes
No
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Review stage
Started
Completed
Data analysis
Yes
Yes
Provide any other relevant information about the stage of the review here (e.g. Funded proposal, protocol not yet finalised).

6. * Named contact.
The named contact acts as the guarantor for the accuracy of the information presented in the register record.
Wei Shen Tan
Email salutation (e.g. "Dr Smith" or "Joanne") for correspondence: 7. * Named contact email.
Give the electronic mail address of the named contact.
wei.tan@ucl.ac.uk
8. Named contact address
PLEASE NOTE this information will be published in the PROSPERO record so please do not enter private information Give the full postal address for the named contact.
Division of Surgery and Interventional Science, University College London, 74 Huntley Street, WC1E 6AU, London, UK
9. Named contact phone number.
Give the telephone number for the named contact, including international dialling code.
+44 (0)7725953115
10. * Organisational affiliation of the review.
Full title of the organisational affiliations for this review and website address if available. This field may be completed as
'None' if the review is not affiliated to any organisation.
University College London
Organisation web address:
11. Review team members and their organisational affiliations.
Give the title, first name, last name and the organisational affiliations of each member of the review team. Affiliation refers to groups or organisations to which review team members belong.
Dr Wei Shen Tan. UCL
Dr Wei Phin Tan. Rush Medical Center
Mrs Mae-Yen Tan. Glasgow University
Professor John Kelly. UCL
Mr Andrew Feber. UCL
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12. * Funding sources/sponsors.
Give details of the individuals, organizations, groups or other legal entities who take responsibility for initiating, managing, sponsoring and/or financing the review. Include any unique identification numbers assigned to the review by the individuals or bodies listed.
UCL Biomedical Research Center, Urology Foundation 13. * Conflicts of interest.
List any conditions that could lead to actual or perceived undue influence on judgements concerning the main topic investigated in the review.
None
14. Collaborators.
Give the name and affiliation of any individuals or organisations who are working on the review but who are not listed as review team members.
15. * Review question.
State the question(s) to be addressed by the review, clearly and precisely. Review questions may be specific or broad. It may be appropriate to break very broad questions down into a series of related more specific questions. Questions may be framed or refined using PI(E)COS where relevant.
To determine the diagnostic value of novel non-commercially available urinary biomarker for the detection of bladder cancer.
16. * Searches.
Give details of the sources to be searched, search dates (from and to), and any restrictions (e.g. language or publication period). The full search strategy is not required,but may be supplied as a link or attachment.
Comprehensive literature search performed using MEDLINE between January 2013 to July 2017 using the following MESH words:
(bladder cancer OR transitional cell carcinoma OR urothelial cell carcinoma) AND (detection OR diagnosis) AND urine AND (biomarker OR assay).
All studies had a minimum of 20 patients in both bladder cancer and control arms and reported sensitivity and/ or specificity and/ or receiver operating characteristics (ROC) curve. All studies are published in English and full text articles available. All conference abstracts, review articles, editorials, comments and letters to the editor were excluded.

17. URL to search strategy.
Give a link to the search strategy or an example of a search strategy for a specific database if available (including the keywords that will be used in the search strategies).
Yes I give permission for this file to be made publicly available 18. * Condition or domain being studied.
Give a short description of the disease, condition or healthcare domain being studied. This could include health and wellbeing outcomes.
Bladder cancer.
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19. * Participants/population.
Give summary criteria for the participants or populations being studied by the review. The preferred format includes details of both inclusion and exclusion criteria.
Patients with bladder cancer which has been confirmed on cystoscopy and histology.
20. * Intervention(s), exposure(s).
Give full and clear descriptions or definitions of the nature of the interventions or the exposures to be reviewed.
All patients would have cystosocopy and a urinary assay test.
21. * Comparator(s)/control.
Where relevant, give details of the alternatives against which the main subject/topic of the review will be compared (e.g.
another intervention or a non-exposed control group). The preferred format includes details of both inclusion and exclusion criteria.
Patients who have had cystoscopy which confirms the absence of bladder cancer.
22. * Types of study to be included.
Give details of the types of study (study designs) eligible for inclusion in the review. If there are no restrictions on the types of study design eligible for inclusion, or certain study types are excluded, this should be stated. The preferred format includes details of both inclusion and exclusion criteria.
All studies must have two groups of patients: patients with bladder cancer and patients without bladder cancer.
23. Context.
Give summary details of the setting and other relevant characteristics which help define the inclusion or exclusion criteria.
24. * Primary outcome(s).
Give the pre-specified primary (most important) outcomes of the review, including details of how the outcome is defined and measured and when these measurement are made, if these are part of the review inclusion criteria.
Diagnostic capability of novel urinary biomarker- sensitivity, specificity, negative predictive value, positive predictive value, area under the curve.
Timing and effect measures
25. * Secondary outcome(s).
List the pre-specified secondary (additional) outcomes of the review, with a similar level of detail to that required for primary outcomes. Where there are no secondary outcomes please state ‘None’ or ‘Not applicable’ as appropriate to the review Determine the diagnostic capability of combination/ multiple urinary biomarkers in comparison to single assay/ urinary biomarker.
Timing and effect measures
26. Data extraction (selection and coding).
Give the procedure for selecting studies for the review and extracting data, including the number of researchers involved and how discrepancies will be resolved. List the data to be extracted.
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Title and abstracts of studies will be retrieved and independently screened by two review authors for studies that potentially meet the inclusion criteria. Additional full text searches will be performed to determine eligibility of studies. Any disagreement will be resolved through discussion with a third reviewer.
Data extracted will include: study population, new or recurrent bladder cancer status, percentage of low grade bladder cancer, diagnostic capability of test (sensitivity, specificity, negative predictive value, positive predictive value, area under the curve), method of processing urinary sample for assay.

27. * Risk of bias (quality) assessment.
State whether and how risk of bias will be assessed (including the number of researchers involved and how discrepancies will be resolved), how the quality of individual studies will be assessed, and whether and how this will influence the planned synthesis.
The Cochrane risk of bias tool will be used. Two review authors will independently assess risk of bias.
28. * Strategy for data synthesis.
Give the planned general approach to synthesis, e.g. whether aggregate or individual participant data will be used and whether a quantitative or narrative (descriptive) synthesis is planned. It is acceptable to state that a quantitative synthesis will be used if the included studies are sufficiently homogenous.
Aggregate patient data will be used. A narrative synthesis of included studies will be included for the study population, new or recurrent bladder cancer status, percentage of low grade bladder cancer. diagnostic capability of test (sensitivity, specificity, negative predictive value, positive predictive value, area under the curve), method of processing urinary sample for assay.
We anticipate that there will be limited use for a meta-analysis because of the range of different novel urinary biomarkers in the literature.
29. * Analysis of subgroups or subsets.
Give details of any plans for the separate presentation, exploration or analysis of different types of participants (e.g. by age, disease status, ethnicity, socioeconomic status, presence or absence or co-morbidities); different types of intervention (e.g.
drug dose, presence or absence of particular components of intervention); different settings (e.g. country, acute or primary care sector, professional or family care); or different types of study (e.g. randomised or non-randomised).
None planned.
30. * Type and method of review.
Select the type of review and the review method from the lists below. Select the health area(s) of interest for your review.
Type of review
Cost effectiveness
No
Diagnostic
Yes
Epidemiologic
No
Individual patient data (IPD) meta-analysis
No
Intervention
No
Meta-analysis
No
Methodology
No
Network meta-analysis
No
Pre-clinical
No
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Prevention
No
Prognostic
No
Prospective meta-analysis (PMA)
No
Qualitative synthesis
No
Review of reviews
No
Service delivery
No

Systematic review
Yes
Other
No
Health area of the review
Alcohol/substance misuse/abuse
No
Blood and immune system
No
Cancer
Yes
Cardiovascular
No
Care of the elderly
No
Child health
No
Complementary therapies
No
Crime and justice
No
Dental
No
Digestive system
No
Ear, nose and throat
No
Education
No
Endocrine and metabolic disorders
No
Eye disorders
No
General interest
No
Genetics
Yes
Health inequalities/health equity
No
Infections and infestations
No
International development
No
Mental health and behavioural conditions
No
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Musculoskeletal
No
Neurological
No
Nursing
No
Obstetrics and gynaecology
No
Oral health
No
Palliative care
No
Perioperative care
No

Physiotherapy
No
Pregnancy and childbirth
No
Public health (including social determinants of health) No
Rehabilitation
No
Respiratory disorders
No
Service delivery
No
Skin disorders
No
Social care
No
Tropical Medicine
No
Urological
Yes
Wounds, injuries and accidents
No
Violence and abuse
No
31. Language.
Select each language individually to add it to the list below, use the bin icon to remove any added in error.
English
There is an English language summary.
32. Country.
Select the country in which the review is being carried out from the drop down list. For multi-national collaborations select all the countries involved.
England
33. Other registration details.
Give the name of any organisation where the systematic review title or protocol is registered (such as with The Campbell Collaboration, or The Joanna Briggs Institute) together with any unique identification number assigned. (N.B. Registration details for Cochrane protocols will be automatically entered). If extracted data will be stored and made available through a https://www.crd.york.ac.uk/PROSPERO/#recordDetails 7/9
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repository such as the Systematic Review Data Repository (SRDR), details and a link should be included here. If none, leave blank.
34. Reference and/or URL for published protocol.
Give the citation and link for the published protocol, if there is one Yes I give permission for this file to be made publicly available 35. Dissemination plans.
Give brief details of plans for communicating essential messages from the review to the appropriate audiences.
Publication in peer review journal.
Do you intend to publish the review on completion?
Yes
36. Keywords.
Give words or phrases that best describe the review. Separate keywords with a semicolon or new line. Keywords will help users find the review in the Register (the words do not appear in the public record but are included in searches). Be as specific and precise as possible. Avoid acronyms and abbreviations unless these are in wide use.
Bladder cancer
Biomarker
Urine
Diagnosis
Systematic review
37. Details of any existing review of the same topic by the same authors.

Give details of earlier versions of the systematic review if an update of an existing review is being registered, including full bibliographic reference if possible.
38. * Current review status.
Review status should be updated when the review is completed and when it is published.
Ongoing
39. Any additional information.
Provide any other information the review team feel is relevant to the registration of the review.
40. Details of final report/publication(s).
This field should be left empty until details of the completed review are available.
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https://www.crd.york.ac.uk/PROSPERO/#recordDetails 9/9

A4: DETECT I IRAS approval

Health Research Authority

North West - Liverpool Central Research Ethics Committee

3rd Floor
Barlow House
4 Minshull Street
Manchester
M1 3DZ
Telephone: 0207 104 8006

09 March 2016

Professor John Kelly
Professor of Uro-Oncology
University College London
Room 447
74 Huntley Street
London
WC1E 6AU

Dear Professor Kelly

Study title: A prospective observational study to determine the negative predictive value of UroMark to rule out the presence of bladder cancer in patients with haematuria.
REC reference: 16/NW/0150
IRAS project ID: 179245

Thank you for your response of 09 March 2016, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Miss Carol Ebenezer, nrescommittee.northwest-liverpoolcentral@nhs.net

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Covering letter on headed paper [REC Cover Letter]		**12 February 2016**
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [UCL insurance]		**04 February 2016**
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		
GP/consultant information sheets or letters [Clean copy of the GP letter]	**1.0**	**26 January 2016**

Letter from funder [MRC letter]		**21 July 2015**
Letters of invitation to participant [Invitation letter]	**1.1**	**08 March 2016**
Letters of invitation to participant [Tracked invitation letter]	**1.1**	**08 March 2016**
Non-validated questionnaire [Health Economics]	**1.1**	**08 March 2016**
Non-validated questionnaire [Tracked HE Questionnaire]	**1.1**	**08 March 2016**
Other [John Kelly's GCP]		**15 April 2015**
Participant consent form [Clean copy of the ICF]	**1.1**	**08 March 2016**
Participant consent form [Tracked ICH]	**1.1**	**08 March 2016**
Participant information sheet (PIS) [Clean copy of the PIS]	**1.1**	**08 March 2016**
Participant information sheet (PIS) [Tracked PIS]	**1.1**	**08 March 2016**
REC Application Form [REC_Form_15022016]		**15 February 2016**
Research protocol or project proposal [Clean copy of Protocol]	**1.0**	**27 January 2016**
Summary CV for Chief Investigator (CI) [Prof Kelly's CV]		**06 December 2015**

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

16/NW/0150 **Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project.

Yours sincerely

[redacted]

Signed on behalf of:
Mrs Julie Brake
Chair

Email:nrescommittee.northwest-liverpoolcentral@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Ms Suzannne Emerton, JRO UCL

A5: DETECT I HRA approval

Health Research Authority

Professor John Kelly
Professor of Uro-Oncology
Email: hra.approval@nhs.net
University College London
Room 447
74 Huntley Street
London
WC1E 6AU

18 May 2016

Dear Professor Kelly,

Letter of HRA Approval for a study processed under pre- HRA Approval systems

Study title: A prospective observational study to determine the negative predictive value of UroMark to rule out the presence of bladder cancer in patients with haematuria.
IRAS project ID: 179245
REC reference: 16/NW/0150
Sponsor: UCL

Thank you for your request to bring the above referenced study under HRA Approval.

I am pleased to confirm that the study has been given **HRA Approval**, on the basis of the document set provided, any clarifications noted in this letter and taking account of reviews and approvals previously conducted and issued.

The extension of HRA Approval to this study on this basis allows the sponsor and NHS organisations to set-up the study in accordance with HRA Approval processes, with decisions on study set-up being taken on the basis of capacity and capability alone.

Participation of NHS Organisations in England
The sponsor should now provide a copy of this letter to participating NHS organisations in England which are being set up in accordance with HRA Approval Processes.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read** Appendix B **carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability.
- Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study.

Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

In addition to the document, *"After Ethical Review – guidance for sponsors and investigators"* , issued with your REC Favourable Opinion, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net. Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/.

Your IRAS project ID is **179245.** Please quote this on all correspondence.

Yours sincerely

Simon Connolly
Senior Assessor

Email: hra.approval@nhs.net

Copy to: Ms Suzannne Emerton, UCL (Sponsor and Lead NHS R&D contact)

A6: DETECT I Patient information sheet

(To be printed on local hospital headed paper)

PATIENT INFORMATION SHEET

DETECT I

A prospective observational study to determine the negative predictive value of UroMark to rule out the presence of bladder cancer in patients with haematuria.

We are inviting you to take part in a research study called DETECT I.

Before you decide whether to take part it is important that you understand why the research is being done and what it will involve. One of your doctors or nurses will go through this information sheet with you and answer any questions you may have. Please take time to read the information carefully and to discuss it with relatives, friends and your GP if you wish.

Please ask if anything is unclear or you need any further information.

Thank you for reading this and considering taking part in our research.

What is the purpose of the DETECT I study?

We are investigating whether UroMark, a test which has been developed to detect bladder cancer cells in urine, can be used to identify patients who have bladder cancer.

Why am I being invited to take part?

You are being invited to take part because you were referred to hospital for tests following the detection of blood in your urine. Blood in urine is called haematuria and your doctor has referred you for further investigations at this hospital. Most patients with haematuria will **not** have bladder cancer but in about 1 in 10 patients a bladder cancer will be detected. We aim to include 889 people like you in this study and we estimate that 80-100 patients will be found to have bladder cancer.

Do I have to take part?

No, it is up to you to decide whether to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. You will be free to withdraw from the study at any time and do not have to give a reason. This will not affect the standard of care you receive.

What is being studied?

We are investigating whether the test called UroMark can detect bladder cancer in patients who are being investigated for haematuria. UroMark is currently in development stage and early results indicate that it can detect bladder cancer with a high degree of certainty. The test detects changes in the DNA of cells which are present in urine. Cancer cells will have changes in DNA, called mutations or epigenetic alterations and we would like to understand if the UroMark test can detect these abnormal cells in a urine sample.

To understand if the UroMark test performs we will compare it to other established urine tests. Currently none of the established tests can be used in clinical practice alone but it will be important to include other tests to understand whether UroMark is more accurate.

It is possible that new tests will be developed by other research groups or industry. We would like to store any surplus material to be tested in future studies involving new urine based tests.

What will happen to me if I take part?

You have been referred to hospital to have tests because of the finding of either visible or non-visible blood in your urine. Everyone who agrees to take part will be receiving the normal investigations for haematuria. Your doctor or clinical nurse specialist will talk to you about the standard tests which all patients receive.

In addition, we will ask you to provide a urine sample, this urine sample will be used for molecular studies and future research if you give consent. We will provide you with a sample collection kit and ask you to send the urine sample from home. The sample collection kit has four containers which are similar to regular urine sample containers. Each container is placed in a postal package and we ask that you send the samples back to us using the stamped addressed mailing package provided. The sample kit comes with written information and clear instructions. The kit can be posted through a Royal Mail post box or post office. A questionnaire will also be given to you to complete at home and can be sent in the same envelope as the urine sample. The questionnaire is a very straight forward one that should only take a maximum of 20 minutes for you to complete. The questionnaire has been devised to record your confidence in providing a urine sample for diagnostic purposes.

The best way to understand whether UroMark is accurate will be to compare the UroMark test result with the standard investigations which you receive. In addition we would like to compare the UroMark test with other urine tests and in a subset of patients we will ask for a second sample of urine to be provided when you are attending the hospital. If a urine sample is not valid to provide any results you may be asked to provide another urine sample in these circumstances.

If you agree to take part, your doctor or nurse will register you with the research centre. The centre will then record your details and the results of any tests which you receive as part of the standard investigation for haematuria.

It is important that you only agree to join the study if you would be completely satisfied to receive the UroMark collection kit and provide a urine sample.

You will be given time to ask all the questions you want.

Will the results of the UroMark test alter treatment?

The UroMark test result will not be known to your doctor or nurse involved with your investigations at your hospital or any subsequent treatment. It is important to be aware that the study is being conducted to understand whether the test will be useful and it will be necessary to have the test results from all 889 patients before we can determine this.

The majority of patients with haematuria do not have bladder cancer and other conditions can cause haematuria. Your doctor and nurse will discuss the results of the investigations which you will have at your hospital and whether any further treatment is necessary.

What will happen to the samples I give?

The samples you provide during this study will be analysed to verify the sensitivity of the Uromark assay. Part of the urine samples that are provided by you will be stored at the BioBank (UCl/UCLH BioBank for Studying Health and Disease) the samples will be used following the human tissue act (HTA) guidelines.

What are the possible benefits of taking part?

The information learned from this study may help us to improve ways to detect and exclude cancer in the future.

What are the possible disadvantages and risks of taking part?

If you agree to take part you will have to provide a urine sample at home and post the sample to the UroMark centre. A stamp addressed envelope has been provided which can be posted from any standard Royal Mail post box or post office.

How will confidentiality be maintained?

Your medical notes will be seen by authorised members of the research team at your hospital, so that they can collect information needed for the DETECT I study. When you join the study, your name, date of birth, postcode, hospital number and NHS or Community Health Index (CHI) number will be passed to the DETECT Clinical Trials Group at University College London where

the study is being coordinated. You will be given a unique registration number, which will be used together with your initials and date of birth on forms that the research staff will use. All information about you will be coded with this registration number and will be stored securely. It will be treated as strictly confidential and nothing that might identify you will be revealed to any third party.

Scientific and medical employees of UCL, and those conducting the study with them or members of regulatory bodies, may need to examine your medical records to ensure the study is being run properly and that the information collected on the forms is correct, but your confidentiality will be protected at all times.

All the information that is sent to the UCL Trials office will be kept for a minimum of 20 years after the DETECT I study has ended.

What happens if I change my mind during the study?

You are free to withdraw from the study at any time. You do not have to give a reason and your future treatment and care will not be affected.

What if something goes wrong?

Every care will be taken in the course of this study. If you are not satisfied with the general care and treatment you receive, please speak first to your doctor, who will try to resolve the problem. If you remain dissatisfied and wish to complain formally about the care and treatment received during the study, you may do so under the standard NHS complaints procedure which is available to you from your study doctor's hospital.

In the unlikely event that you are injured by taking part, compensation may be available. If you are harmed due to the negligence of someone treating you, then you may have grounds for legal action for compensation. NHS Trusts are responsible for clinical negligence and other negligent harm to individuals that are under their care and covered under the NHS Indemnity Scheme.

What will happen to the results of the research study?

Independent experts will review the progress of the research, and the results will be published in a respected medical journal once we are sure they are reliable. No information that could identify you will be included and you will not be identified in any report or publication.

We will summarise the results for participants once they are available. Your hospital will be able to give you a copy.

Who is organising and funding the research?

DETECT I is organised by University College London and University College London Hospital (Chief Investigator: Professor John Kelly). The research is approved and funded by the Medical Research Council.

Who has reviewed the study?

All research in the NHS is looked at by an independent group of people, called a Research Ethics Committee, to protect participants' safety, rights, wellbeing and dignity. DETECT I has been reviewed and approved by North West – Liverpool Central Research Ethics Committee on behalf of all hospitals throughout the UK.

What happens now?

You will have some time to think about the study and make your decision. Your doctor or nurse will be happy to answer any questions. You may wish to discuss it with your family or friends. Once you have reached your decision please let your doctor or nurse know.

You will be asked to sign a consent form and be given a copy to keep together with this information sheet. Your GP will be told that you are taking part in the DETECT I study. If at any time you have any questions about the study you should contact your hospital consultant.

Contact details

If at any time you have any questions about the study you should contact your local study team:

Local consultant's name: XXXX

Local research registrar: XXXX

Address: XXXX

Tel: XXXX

Email: XXXX

Any questions about the research, your rights as a patient, or any complaints should be handled by speaking with a research doctor from the study team. If you are not satisfied or do not wish to discuss the matter with a doctor from the study team, you can speak to the hospitals Patient Advise and Liaison Services (PALS):

Patient Advice and Liaison Services

Telephone number: 020 3447 3042

Email: PALS@uclh.nhs.uk

Thank you for your interest in our research.

A7: DETECT I Patient consent form

INFORMED CONSENT FORM

DETECT I

A prospective observational study to determine the negative predictive value of UroMark to rule out the presence of bladder cancer in patients with haematuria.

REC ref: 16/NW/0150

Please initial box

I confirm that I have read and understood the DETECT I Patient Information Sheet version 1.1 dated 08/03/2016 for the above study and have had the opportunity to ask questions.	☐
I agree to take part in the DETECT I study once all tests confirm I am suitable to participate. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I agree to my name, date of birth, postcode, hospital number and NHS or Community Health Index (CHI) number being sent to Clinical Trials Group UCL when I join DETECT I.	☐
I understand that sections of any of my medical notes may be looked at by responsible individuals from the research team, from regulatory authorities, from the NHS Trust where it is relevant to my taking part in the research. I give permission for these individuals to have access to my records.	☐
I give permission for my name to be used to obtain information about my health status from records held by the NHS and maintained by the NHS Information Centre and the NHS Central Register or any applicable NHS information system (including linkage to routine hospital admission data).	☐
I give this consent solely so that researchers may follow up on my health status after my participation in the study.	☐
I grant advance authorisation for the molecular study on the samples (urine) I provide and possible future sharing of information collected about me with other organisations, with the understanding that I will not be identifiable from this information (optional).	☐
I grant advance authorisation for molecular studies on the samples (urine) I provide and possible future research on my stored samples, with the understanding that I will not be identifiable from these samples. I understand that that prior approval of an ethics committee will be obtained (optional).	☐

Name of Patient	Date	Signature
Name of person taking consent	Date	Signature

A8: DETECT I Clinical record form

DETECT I

SUBJECT NUMBER

DTA	SN	SN	SN	NUM	NUM	NUM	LET

DATE OF ASSESSMENT:_____/_____/_____ (dd/mm/yy)

INCLUSION CRITERIA

	YES	NO
Patient must be over 18 years of age.	☐	☐
Upper tract scan has to be within 4 weeks of patient being registered.	☐	☐
Patient undergoing investigation for visible and non-visible haematuria.	☐	☐
Able to give informed written consent to participate.	☐	☐

EXCLUSION CRITERIA

	YES	NO
Unwilling to have standard haematuria investigations.	☐	☐
Unable to give informed consent.	☐	☐

PATIENT ENROLMENT/REGISTRATION

Investigator's Name:

_____

Subject Trial Number Allocated DTA SN SN SN NUM NUM NUM LET

Date of Enrolment/Registration __/_____/_____(dd/mm/yy)

Subject Identifier

Surname Initial	First Name—1st letter	First name—2nd letter

INFORMED CONSENT

DATE INFORMED CONSENT TAKEN	D D M M Y Y
DOB	D D M M Y Y
GENDER	MALE / FEMALE
SITE	

WITHDRAW OF CONSENT (IF APPLICABLE) ☐

DATE OF WITHDRAWAL OF CONSENT

REASON OF WITHDRAWAL OF CONSENT:

_____/_____/_____(dd/mm/yy)

_____

Version Number: 2.0 dated 15 Aug 2016
Version Number: 2.0 dated 15 Aug 2016

DETECT I

SUBJECT NUMBER	DTA	SN	SN	SN	NUM	NUM	NUM	LET

DEMOGRAPHIC DATA					
ETHINICITY:					
White	☐	White British	☐	White Other	☐
Mixed Race	☐	White & Black Caribbean	☐	White & Asian	☐
Asian or Asian British	☐	Indian	☐	Pakistani	☐
Black or Black British	☐	Caribbean	☐	Bangladeshi	☐
Chinese or other ethnicity	☐	Chinese	☐	African	☐
White Irish	☐	Other (please specify)	☐	Black Other	☐

OCCUPATION DATA			
What is your employment status?			
Full time paid or self employed	☐	Home maker	☐
Part time or self-employed	☐	Retired	☐
Voluntary work	☐	Exempt through disability	☐
Unemployed	☐	Other (please specify)	☐
Student	☐	What is their occupation _____	
Has the patient ever worked as a gardener, painter , hairdresser/ barber, textile worker or worked in a aluminium factory?	No ☐	Yes (if Yes, please specify) ☐ _____	

Version Number: 2.0 dated 15 Aug 2016

DETECT I

SUBJECT NUMBER	DTA	SN	SN	SN	NUM	NUM	NUM	LET

INVESTIGATIONS

UROMARK ASSAY	
Date UroMark Kit given to Patient	D D M M Y Y
CONTROL URINE SAMPLE	
Date the Void Urine Sample Was Taken	D D M M Y Y
HEALTH ECONOMICS	
Date Questionnaire Given to Patient	D D M M Y Y

Was Cytology or any other Urinary Biomarker Performed on Urine Sample

Yes	☐
No	☐
If Yes, which urinary biomarker	
Cytology	☐
NMP22	☐
FISH	☐
Other	☐ _____
Urinary biomarker result	
Positive	☐
Negative	☐
Atypical	☐
Indeterminate	☐
Other	☐ _____

SUBJECT NUMBER | DTA | INT | INT | INT | NUM | NUM | NUM | LET |

Flexible Cystoscopy Findings		
Date of Flexible Cystoscopy	D D M M Y Y	
Tumour Present (Visual)	Yes ☐	No ☐
Tumour Size (Visual)	_____cm	
Number of Tumours (Visual)	_____	

Other Cystoscopy Findings	
Red patch requiring biopsy	☐
Bladder Stone	☐
Cystitis (UTI)	☐
Prostate / Bladder bleeding	☐
No Abnormality	☐
Other (please specify) _____	☐

DETECT I

SUBJECT NUMBER | DTA | SN | SN | SN | NUM | NUM | NUM | LET |

IMAGING RESULTS (Can be more than One)

	Date of Imaging	Results	Yes	No
Ultrasonography (USS)	D D M M Y Y	Normal	☐	☐
		Bladder TCC	☐	☐
		Bladder Stone	☐	☐
NA ☐		Bladder Filling Defect	☐	☐
		Renal RCC	☐	☐
		Upper Tract TCC	☐	☐
		Upper Tract Stone	☐	☐
		Prostate Defect	☐	☐
		Other (please specify)	☐	☐
		_____		
CT KUB	D D M M Y Y	Normal	☐	☐
		Bladder TCC	☐	☐
		Bladder Stone	☐	☐
NA ☐		Bladder Filling Defect	☐	☐
		Renal RCC	☐	☐
		Upper Tract TCC	☐	☐
		Upper Tract Stone	☐	☐
		Prostate Defect	☐	☐
		Other (please specify)	☐	☐
		_____		
CT Urogram	D D M M Y Y	Normal	☐	☐
		Bladder TCC	☐	☐
		Bladder Stone	☐	☐
NA ☐		Bladder Filling Defect	☐	☐
		Renal RCC	☐	☐
		Upper Tract TCC	☐	☐
		Upper Tract Stone	☐	☐
		Prostate Defect	☐	☐
		Other (please specify)	☐	☐
		_____		
MRI	D D M M Y Y	Normal	☐	☐
		Bladder TCC	☐	☐
		Bladder Stone	☐	☐
NA ☐		Bladder Filling Defect	☐	☐
		Renal RCC	☐	☐
		Upper Tract TCC	☐	☐
		Upper Tract Stone	☐	☐
		Prostate Defect	☐	☐
		Other (please specify)	☐	☐
		_____		

SUBJECT NUMBER	DTA	SN	SN	SN	NUM	NUM	NUM	LET

Did imaging pick up bladder abnormality	Yes ☐	No ☐
Did imaging suggest bladder tumour	Yes ☐	No ☐

AT TURBT OR BIOPSY CYSTOSCOPY

*If Tumour is Present

DATE OF TURBT	D D M M Y Y

HISTOLOGY				
☐ TCC	☐ Adenocarcinoma	☐ Squamous carcinoma	☐ Normal	☐ Other: _____

GRADE	1 ☐	2 ☐		3 ☐
STAGE	pTa ☐	pT1 ☐	≥pT2 ☐	Isolated CIS ☐ Concurrent CIS ☐
NUMBER OF TUMOURS				
SIZE	_____cm			
COMMENTS				

A9: DETECT II IRAS approval

Health Research Authority
London - Stanmore Research Ethics Committee

Ground Floor
NRES/HRA
80 London Road
London

SE1 6LH

Telephone: 020 7972 2554

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

20 July 2016 re-issued 11/08/2016

Prof John Kelly
Professor of Uro-oncology
Division of Surgery and Interventional Science, University College London
74 Huntley Street
London
WC1E 6AU

Dear Prof Kelly
Study title: A multi-centre observational study design to determine the sensitivity of the UroMark assay, a urine test, to detect new and recurrent low, intermediate and high grade bladder cancer.
REC reference: 16/LO/1044
IRAS project ID: 203022
Thank you for your letter of 1st July responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Ms Julie Kidd, nrescommittee.london-stanmore@nhs.net.
Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

You should notify the REC once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. Revised documents should be submitted to the REC electronically from IRAS. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which you can make available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.
Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.
Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).
Guidance on applying for NHS permission for research is available in the Integrated Research Application System**, www.hra.nhs.uk** or at **http://www.rdforum.nhs.uk.**

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations
Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett **(catherineblewett@nhs.net)**, the HRA does not, however, expect exceptions to be made.
Guidance on where to register is provided within IRAS.
It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites
The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites
Approved documents
The final list of documents reviewed and approved by the Committee is as follows:

Document	Version	Date
Covering letter on headed paper [REC Cover Letter]		**17 May 2016**
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only)		**13 July 2015**
IRAS Application Form [IRAS_Form_18052016] 2016		**17th May 2016**
Letter from funder [MRC Letter]		**21 July 2015**

Letters of invitation to participant [Invitation letter]	**1.0**	**07 March 2016**
Other [CI GCP]		**15 April 2015**
Other [Independent External Review]		
Other [Email trail not suitable PR]		**20 May 2016**
Other [Protocol]	**1.1**	**28 June 2016**
Participant consent form [ICF]	**1.1**	**28 June 2016**
Participant information sheet (PIS) [PIL]	**1.1**	**28 June 2016**
Summary CV for Chief Investigator (CI)		

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review
Reporting requirements

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

Notifying substantial amendments
Adding new sites and investigators
Notification of serious breaches of the protocol
Progress and safety reports
Notifying the end of the study
The HRA website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.
User Feedback
The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/
HRA Training
We are pleased to welcome researchers and R&D staff at our training days – see details at http://www.hra.nhs.uk/hra-training/
16/LO/1044 Please quote this number on all correspondence With the Committee's best wishes for the success of this project.

Yours sincerely
PP

[redacted] Rosemary Hill
Chair
Email:nrescommittee.london-stanmore@nhs.net
Enclosures: "After ethical review – guidance for researchers"
Copy to: Ms Suzanne Emerton, JRO UCL

A10: DETECT II HRA approval

Health Research Authority

Professor John Kelly

Email: hra.approval@nhs.net

Professor of Uro-oncology
Division of Surgery and Interventional Science, University College London
74 Huntley Street
London
WC1E 6AU

30 August 2016

Letter of HRA Approval

Dear Professor Kelly

Study title: A multi-centre observational study design to determine the sensitivity of the UroMark assay, a urine test, to detect new and recurrent low, intermediate and high grade bladder cancer.
IRAS project ID: 203022
REC reference: 16/LO/1044
Sponsor: University College London
I am pleased to confirm that HRA Approval **has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.**
Participation of NHS Organisations in England
The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read** Appendix B **carefully**, in particular the following sections:
Participating NHS organisations in England – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
Confirmation of capacity and capability - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability.
Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria) - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.
Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from *www.hra.nhs.uk/hra-approval.*
Appendices
The HRA Approval letter contains the following appendices:
A – List of documents reviewed during HRA assessment
B – Summary of HRA assessment

After HRA Approval
The document "After Ethical Review – guidance for sponsors and investigators", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:
Registration of research
Notifying amendments
Notifying the end of the study
The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:
HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the After Ethical Review document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the *HRA website,* and emailed to hra.amendments@nhs.net
The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the *HRA website.*
Scope
HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/
If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback
The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please email the HRA at *hra.approval@nhs.net.* Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.
HRA Training
We are pleased to welcome researchers and research management staff at our training days – see details at *http://www.hra.nhs.uk/hra-training/*

Your IRAS project ID is **203022**. Please quote this on all correspondence.

Yours sincerely

Miss Helen Penistone
Assessor

Email: hra.approval@nhs.net
Copy to: Mr Onyike Nmaju, UCL, (sponsor contact) randd@uclh.nhs.uk

Ms Suzanne Emerton, JRO UCL(lead NHS R&D contact) randd@uclh.nhs.uk

NIHR CRN Portfolio Applications Team

A11: DETECT II patient information sheet

(To be printed on local hospital headed paper)

PATIENT INFORMATION SHEET

DETECT II

A multicentre observational study design to determine the sensitivity of the UroMark assay; a urine test to detect new and recurrent low, intermediate and high grade bladder cancer.

You are being invited to take part in a research study called DETECT II. Before you decide, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information carefully.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the DETECT II study?

DETECT II is a study examining a test called UroMark which has been developed as a test to detect bladder cancer cells in urine. The purpose of the DETECT II study is to evaluate how well the UroMark test can detect a variety of different bladder tumours. We will also evaluate if the UroMark test can detect recurrence of bladder cancer in patients undergoing surveillance cystoscopy.

Why am I being invited to take part?

You are being invited to take part because your recent cystoscopy suggests that there is an abnormal area in the bladder which will require further investigation/ treatment. We would like to obtain a urine sample for research purposes to determine if the UroMark test is comparable to cystoscopy.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part, you are still free to withdraw at any time and without providing a reason. This will not affect the standard of care you receive.

What is being studied?

We are investigating whether the test called UroMark can detect bladder cancer in a urine sample. UroMark is currently in development stage and early results indicate that it can detect bladder cancer with a high degree of certainty. The test detects changes in the DNA of cells which are present in urine. Cancer cells will have changes in DNA, called ‘mutations’ or ‘epigenetic alterations’ and we would like to understand if the UroMark test can detect these abnormal cells in a urine sample. We aim to recruit about 400 patients with bladder cancer of varying grade and stage.

We will compare the UroMark test to other tests which may be able to detect cancer. It will be important to include other tests to understand whether UroMark is more accurate.

This study is also to asses if the UroMark can be used to detect recurrence of bladder cancer. Biomarkers in the urine will determine which patients have cancer.

We will also assess your perspective on cystoscopy and the use of a urine test to detect bladder cancer during surveillance using a questionnaire. Selected patients will be invited for a telephone interview to explore their experience of being diagnosed with bladder cancer, having cystoscopy and using a urine test for surveillance. The telephone interview will be recorded. All recordings will be anonymised to maintain your confidentiality and therefore patient identifiable details will not be recorded

Patients who test positive will be recommended and likely to have bladder cancer and will be referred for cystoscopy, and those who test negative will be discharged. For patients undergoing surveillance for recurrent disease they will be referred for cystoscopy if UroMark is positive, or re-tested at the appropriate interval. This could potentially avoid or reduce the need for cystoscopy every few months, which is the current practice. It is possible that new tests will be developed by other research groups or industry. Therefore, we would like to store any surplus samples to be used in future studies involving new urine based tests. These molecular studies would involve using DNA in your urine.

What will happen to me if I take part?

Everyone who agrees to take part will be receiving the normal investigations and treatment for bladder cancer as well as the routine follow up to detect a recurrence of bladder cancer. Your doctor or clinical nurse specialist will talk to you about the standard tests, treatment and the follow-up for bladder cancer which all patients receive.

Over a 24 month period, all patients will have routine surveillance cystoscopy at various intervals dependent on type of cancer as part of standard clinic practice. Any recurrent bladder cancer will be treated according to standard practice.

If you agree to take part, the research team will register you and record your clinical and contact details as well as any relevant test results. You will be provided a UroMark urine collection kit to allow you to collect your urine sample any time from **48 HOURS after your cystoscopy**. All provided bottles should be filled up. You can collect the sample at any time and can either fill up all three tubes at one go or mix urine samples from different times you pass urine to fill up the tubes. The urine collected can be posted through a Royal Mail post box or post office. Clear instructions are provided in each kit.

You will also be sent a urine collection kit at three monthly intervals via post over a 2-year period. These samples should ideally be collected before you have your next surveillance cystoscopy.

During the surveillance period, you will be sent a questionnaire via post to assess your perspective on cystoscopy and the use of a urine test to detect bladder cancer. Some patients will be invited for a telephone interview to explore their experience of being diagnosed with bladder cancer and having cystoscopy and using a urine test for surveillance.

What if new information becomes available?

Sometimes during the course of a research project, if new information becomes available about any aspect of the study you will be informed about it. As this study is not changing your treatment you will not have to worry. If any new information is made available that could affect you, your doctor will notify you and discuss with you whether you want to continue in the study. If you decide to withdraw, your research doctor will make arrangements for your care to continue. If you decide to continue in the study, you will be asked to sign an updated consent form (if applicable).

Will the results of the UroMark test alter treatment?

The UroMark test results will **NOT** alter your treatment. The UroMark test result will not be known to your doctor or nurse involved with your investigations at your hospital or any subsequent treatment. This is because the UroMark is still considered experimental and you would have already received cystoscopy which is the current gold standard test.

What will happen to the samples I give?

The samples you provide during this study will be analysed to verify the sensitivity of the UroMark assay. The remainder of the urine samples that are provided by you will be stored anonymously at the BioBank (University College London (UCL) / UCL Hospital (UCLH) BioBank for Studying Health and Disease) and the samples will be used in accordance to the Human Tissue Act (HTA) guideline.

What are the possible benefits of taking part?

The information learned from this study may help us to improve ways to detect and exclude cancer in the future. The results of this study will help us to design new ways to monitor patients with bladder cancer. In the future it may be possible to reduce the number of surveillance cystoscopies.

What are the possible disadvantages and risks of taking part?

There are no disadvantages or risks in taking part in this study as it is an observational study and your treatment would be standard of care. You will be asked to provide a urine sample at 3 monthly intervals during this study.

How will confidentiality be maintained?

Scientific and medical employees of UCL, and those conducting the study as well as members of regulatory bodies, may need to examine your medical records to ensure the study is being run according to protocol and that the information collected on the forms is accurate, but your confidentiality will be protected at all times.

If you consent to take part in the research, your medical records may be inspected by the sponsor for the research which is UCL, for purposes of analysing the results. Your information may also be viewed by people from the UCL and from regulatory authorities to verify that the study is being carried out in accordance to protocol. Any identifiable information that you provide, however, will not be disclosed outside the hospital/GP surgery or the Surgical & Interventional Trials Unit. All information which is collected about you during the course of the study will be kept strictly confidential. Any information about you which leaves the hospital will have your name and address removed.

Your medical notes will be seen by authorised members of the research team at your hospital in order to collect information needed for the DETECT II study. When you join the study, your personal details will be pseudo-anonymised and you will be given a trial number that would be used when we pass your data onto the sponsor and coordinating DETECT II team at UCL where the study is being coordinated. You will be given a unique trial number, which will be used together with a subject identifier on forms. All information about you will be coded with this trial number and will be stored securely in locked cabinets. Again, your information will be treated as strictly confidential and no information that might identify you will be revealed to any third party. All the information that is sent to the UCL Trials Office will be kept for 10 years after the DETECT II study has ended.

What happens if I change my mind during the study?

You are free to withdraw from the study at any time. You do not have to give a reason and your future treatment and care will not be affected.

What if something goes wrong?

Every care will be taken in the course of this study. If you are not satisfied with the general care and treatment you receive, please speak first to your doctor, who will try to resolve the problem. If you remain dissatisfied and wish to complain formally about the care and treatment received during the study, you may do so under the standard NHS complaints procedure which is available to you from your study doctor's hospital.

To find out about it, ask a member of staff, look on the hospital website or contact the Patient Advice and Liaison Service (PALS).

In the unlikely event that you are injured by taking part, compensation may be available. If you are harmed due to the negligence of someone treating you, then you may have grounds for legal action for compensation. NHS Trusts are responsible for clinical negligence and other negligent harm to individuals that are under their care and covered under the NHS Indemnity Scheme.

What will happen to the results of the research study?

A Global Trials Steering Committee has been appointed, this team of independent experts will review the progress of the research, and the results will be published in a respected medical journal once we are sure they are reliable. No information that could identify you will be included and you will not be identified in any report or publication.

We will summarise the results for participants once they are available. Your study team will send you a copy of the results upon request. This study has been placed on an internet directory of clinical trials (www.clinicaltrials.gov) and the result, once available will be posted here.

Who is organising and funding the research?

DETECT II is organised by UCL (Chief Investigator: Professor John Kelly). The research is approved and funded by the Medical Research Council.

Who has reviewed the study?

All research in the NHS is reviewed by an independent group of people, called a Research Ethics Committee, to protect participants' safety, rights, wellbeing and dignity. DETECT II has been reviewed and approved by London – Stanmore Research Ethics Committee.

What happens now?

You will have some time to think about the study and make your decision. Your doctor or nurse will be happy to answer any questions. You may wish to discuss it with your family or friends. Once you have reached your decision please let your doctor or nurse know.

You will be asked to sign a consent form and be given a copy to keep together with this information sheet. If at any time you have any questions about the study you should contact your hospital consultant.

Contact details

If at any time you have any questions about the study you should contact your local study team:

Local study team’s contact details:

[Insert Contact Details]

In an emergency it is best to contact your local GP or go to your local casualty department or dial 999 for an ambulance.

Thank you for your interest in our research study.

A12: DETECT II patient consent form

(To be printed on local hospital headed paper)

INFORMED CONSENT FORM

DETECT II

A multicentre observational study design to determine the sensitivity of the UroMark assay, a urine test, to detect new and recurrent low, intermediate and high grade bladder cancer.

	Please initial box
I confirm that I have read and understood the DETECT II Patient Information Sheet version 2.0 dated 18/10/2016 for the above study and have had the opportunity to ask questions.	☐
I agree to take part in the DETECT II study. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
I agree that my name and contact details will be held by the Surgical & Interventional Trials Unit (University College London). This information will be used for sending sample bottles directly to me.	☐
I understand that sections of any of my medical notes may be looked at by responsible individuals from the research team, regulatory authorities, from the NHS Trust (NHS Health Boards, Scotland) where it is relevant to my taking part in the research. I give permission for these individuals to have access to my records.	☐
I grant authorisation for the molecular study on the samples (urine) I provide and possible future sharing of information collected about me with other organisations, with the understanding that I will not be identifiable from this information.	☐
I understand that I will be sent a questionnaire to assess my perspectives on cystoscopy and a urine test to detect bladder cancer.	☐
I agree to be contacted for a telephone interview to explore my experience of being diagnosed with bladder cancer, having cystoscopy and using a urine test for surveillance. The interview will be recorded and all recordings will be anonymised to maintain confidentiality. *(optional).*	☐
I grant advance authorisation for molecular studies on the samples (urine) I provide and for future research on my stored samples, with the understanding that I will not be identifiable from these samples. I understand that that prior approval of an ethics committee will be obtained *(optional).*	☐

.....	.....	.....
Name of Patient	Date	Signature
.....	.....	.....
Name of person taking consent	Date	Signature

When completed: 1 copy for participant; 1 copy for researcher site file; 1 copy to be kept in medical notes.

A13 DETECT II baseline CRF

DETECT II

SUBJECT NUMBER	DET	SN	SN	SN	NUM	NUM	NUM	LET

CONTACT DETAILS OF PATIENT	
ADDRESS LINE 1:	_____
ADDRESS LINE 2:	_____
CITY:	_____
POST CODE:	☐☐☐☐ ☐☐☐
PHONE NUMBER	_____
EMAIL	_____

SMOKING HISTORY		
HAS THE PATIENT EVER SMOKED	Yes ☐	No ☐
SMOKING STATUS	Current smoker ☐	Ex-smoker ☐
APPROXIMATE NUMBER OF CIGARETTES (OR EQUIVALENT) PER DAY	☐☐☐	
APPROXIMATE LENGTH OF TIME SMOKED FOR (YEARS)	☐☐☐	

UROMARK ASSAY	
DATE UROMARK KIT GIVEN TO PATIENT	D D M M Y Y

TRIAL NUMBER	DET	INT	INT	INT	NUM	NUM	NUM	LET

DEMOGRAPHIC DATA					
ETHINICITY:					
White	☐	White British	☐	White Other	☐
Mixed Race	☐	White & Black Caribbean	☐	White & Asian	☐
Asian or Asian British	☐	Indian	☐	Pakistani	☐
Black or Black British	☐	Caribbean	☐	Bangladeshi	☐
Chinese or other ethnicity	☐	Chinese	☐	African	☐
White Irish	☐	Other (please specify)	☐	Black Other	☐

OCCUPATION DATA			
Full time paid or self employed	☐	Home maker	☐
Part time or self-employed	☐	Retired	☐
Voluntary work	☐	Exempt through disability	☐
Unemployed	☐	Other (please specify)	☐
Student	☐	What is their occupation _____	
Has the patient ever worked as a gardener, painter , hairdresser/ barber, textile worker or worked in a aluminium factory?	No ☐	Yes (if Yes, please specify) ☐ _____	

SUBJECT NUMBER	DET	INT	INT	INT	NUM	NUM	NUM	LET

CYSTOSCOPY

FLEXIBLE CYSTOSCOPY FINDINGS AT ENTRY	
DATE OF FLEXIBLE OR RIGID CYSTOSCOPY	D D M M Y Y
DATE OF SUSPECTED TUMOUR	D D M M Y Y
TUMOUR	New Tumour ☐ Recurrent Tumour ☐
TUMOUR SIZE (IF KNOWN)	_____cm
NUMBER OF TUMOURS (IF KNOWN)	_____

INVESTIGATIONS

Was Cytology or any other urinary biomarker performed

YES		☐
NO		☐
IF YES, WHICH TEST	Cytology	☐
	NMP22	☐
	FISH	☐
	Other	
RESULTS	Positive	☐
	Negative	☐
	Indeterminate	☐
	Other	

SUBJECT NUMBER	DET	SN	SN	SN	NUM	NUM	NUM	LET

TO BE COMPLETED AT TURBT/ BLADDER BIOPSY

PROCEDURE PERFORMED	
TURBT	☐
BLADDER BIOPSY	☐
CYSTODIATHERMY/ LASER ABLATION	☐
CYSTOSCOPIC FINDINGS	
DATE OF TURBT/ BLADDER BIOPSY	D D M M Y Y
TUMOUR SIZE (IF KNOWN)	_____cm
NUMBER OF TUMOURS (IF KNOWN)	_____

HISTOLOGY				
TCC ☐	Adenocarcinoma ☐	Squamous carcinoma ☐	Benign urothelium ☐	Other: _____
GRADE	1 ☐	2 ☐		3 ☐
STAGE	pTa ☐	pT1 ☐	≥pT2 ☐	Isolated CIS ☐
	Concurrent CIS ☐			
HISTOLOGY COMMENTS INCLUDING ANY UNUSUAL VARIANT DETAILS	MICROPAPILLARY ☐ SARCOMATOID ☐ SQUAMOUS DIFFERENTIATION/ METAPLASIA ☐ GLANDULAR DIFFERENTIATION ☐ SMALL CELL ☐ OTHER, PLEASE SPECIFY: _____ _____			

SUBJECT NUMBER	DET	SN	SN	SN	NUM	NUM	NUM	LET

PLANNED FORWARD MANAGEMENT :

SURVEILLANCE CYSTOSCOPY ONLY	YES ☐
PLANNED REPEAT RESECTION	YES ☐
COMMENCE BCG	YES ☐
MITOMYCIN C	YES ☐
HYPERTHERMIA + MITOMYCIN C	YES ☐
EMDA + MITOMYCIN C	YES ☐
CYSTECTOMY	YES ☐
OTHER (PLEASE SPECIFY) _____ _____	YES ☐
NOTES	

A14 DETECT II surveillance CRF

DETECT II

SUBJECT NUMBER	DET	SN	SN	SN	NUM	NUM	NUM	LET

FOLLOW UP (TO BE COMPLETED FOR ALL CYSTOSCOPY INTERVALS)

VISIT (MONTHS) *please circle:* 3 6 9 12 15 18 21 24

FLEXIBLE CYSTOSCOPY			
WAS CYTOLOGY OR ANY OTHER URINARY BIOMARKER PERFORMED	YES ☐	NO ☐	
If yes, which test	Cytology ☐ NMP22 ☐ FISH ☐ Other _____ _____		
RESULTS	Positive ☐ Negative ☐ Indeterminate ☐		
DATE OF FLEXI OR RIGID CYSTOSCOPY	D D M M Y Y		
CYSTOSCOPY FINDINGS	No Tumour ☐	Possible Recurrent Tumour ☐	Other findings ☐
OTHER CYSTOSCOPY FINDINGS:	RED PATCH REQUIRING BIOPSY ☐ BLADDER STONE ☐ CYSTITIS PROSTATE BLEEDING ☐ OTHER _____ ☐		
IF POSSIBLY RECURRENCE:			
DATE OF BIOPSY / TURBT	D D M M Y Y		
NUMBER OF TUMOURS	_____		
SIZE (IF KNOWN)	_____ cm		
IF NO RECURRENCE (CIRCLE THE APPROPRIATE):			
CYSTOSCOPY IN 3, 6, 9, 12 MONTHS OR DISCHARGE			

DETECT II

TO BE COMPLETED AT TURBT/ BLADDER BIOPSY (AT FOLLOW UP)

PROCEDURE PERFORMED	
TURBT	☐
BLADDER BIOPSY	☐
CYSTODIATHERMY/ LASER ABLATION	☐
CYSTOSCOPIC FINDINGS	
DATE OF TURBT/ BLADDER BIOPSY	D D M M Y Y
TUMOUR SIZE (IF KNOWN)	_____cm
NUMBER OF TUMOURS (IF KNOWN)	_____

HISTOLOGY				
TCC ☐	Adenocarcinoma ☐	Squamous carcinoma ☐	Benign urothelium ☐	Other: _____
GRADE	1 ☐	2 ☐		3 ☐
STAGE	pTa ☐	pT1 ☐	≥pT2 ☐	Isolated CIS ☐
	Concurrent CIS ☐			
COMMENTS INCLUDING ANY UNUSUAL VARIANT DETAILS	MICROPAPILLARY ☐ SARCOMATOID ☐ SQUAMOUS DIFFERENTIATION/ METAPLASIA ☐ GLANDULAR DIFFERENTIATION ☐ SMALL CELL ☐ OTHERS: _____			

NEXT CYSTOSCOPY (CIRCLE THE APPROPRIATE)
CYSTOSCOPY IN 3, 6, 9, 12 MONTHS OR DISCHARGE

SUBJECT NUMBER	DET	SN	SN	SN	NUM	NUM	NUM	LET

PLANNED FORWARD MANAGEMENT :

SURVEILLANCE CYSTOSCOPY ONLY	YES ☐
PLANNED REPEAT RESECTION	YES ☐
COMMENCE BCG	YES ☐
MITOMYCIN C	YES ☐
HYPERTHERMIA + MMC	YES ☐
EMDA + MMC	YES ☐
CYSTECTOMY	YES ☐
OTHER (PLEASE SPECIFY) _____	YES ☐
COMMENTS	

A15: DETECT II patient perspectives questionnaire

DETECT II

SUBJECT NUMBER	DET	SN	SN	SN	NUM	NUM	NUM	LET

PATIENT INITIALS | | |

PATIENT DOB | D | D | M | M | Y | Y |

GENDER MALE / FEMALE

HOME POSTCODE | | | | | | | |

LOCAL HOSPITAL _____

DETECT II STUDY

PATIENTS PRESPECTIVES ON CYSTOSCOPY AND THE USE OF A URINARY TEST TO DETECT BLADDER CANCER IN THE SURVEILANCE SETTING

DATE YOU COMPLETED THIS QUESTIONNAIRE:

D	D	M	M	Y	Y

For patient completion

Thank you for agreeing to complete this questionnaire. We will be asking you questions regarding your views regarding cystoscopy and using a urinary test to detect bladder cancer

The questions require you to either tick the selected box or circle a number on the scale.

Cystoscopy is a procedure used to visually inspect the bladder for the presence or absence of tumour. A urinary test involves urinating into a container. You would have had a cystoscopy and used a urinary test before completing this questionnaire

Version Number: 1.0 dated 3rd Oct 2016

DETECT II

PATIENT INITIALS | | | PATIENT DOB | D | D | M | M | Y | Y |

HIGHEST EDUCATION			
High school	☐	GCSE	☐
A Levels	☐	University degree	☐
Higher degree	☐		

PREVIOUS EXPERIENCE WITH CYSTOSCOPY			
Number of previous cystoscopies experienced	≤2 ☐	2-5 ☐	>5 ☐

COMPLICATIONS EXPERIENCED FOLLOWING CYSTOSCOPY		
Blood in urine	Yes ☐	No ☐
Stinging/ burning/ other urinary symptoms when passing urine	Yes ☐	No ☐
Urine infection requiring antibiotics	Yes ☐	No ☐
Other complications		

Overall experience with flexible cystoscopy				
No symptoms		Neutral		Severe symptoms
1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

Pain during flexible cystoscopy				
Painless		Some what painless		Very painful
1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

Anxiety preceding flexible cystoscopy				
Not anxious		Some what anxious		Very anxious
1 ☐	2 ☐	3 ☐	4 ☐	5 ☐

DETECT II

PATIENT INITIALS			PATIENT DOB	D	D	M	M	Y	Y

Neither cystoscopy nor a urinary test will be able to detect all bladder cancers. Cystoscopy will detect approximately 98 of every 100 bladder cancers.

Recurrence is common in bladder cancer and up to 40% of patients can develop recurrence within 5 years

1) What do you think is the risk of your bladder cancer recurring or becoming more aggressive ?

Low ☐	Intermediate ☐	High ☐

2) If the urinary test detects will miss 15 of 100 bladder cancers (vs miss 2 of every 100 cancers in cystoscopy), would you prefer the urinary test or cystoscopy?

Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

3) If the urinary test detects will miss 10 of 100 bladder cancers (vs miss 2 of every 100 cancers in cystoscopy), would you prefer the urinary test or cystoscopy?

Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

4) If the urinary test detects will miss 8 of 100 bladder cancers (vs miss 2 of every 100 cancers in cystoscopy), would you prefer the urinary test or cystoscopy?

Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

5) If the urinary test detects will miss 6 of 100 bladder cancers (vs miss 2 of every 100 cancers in cystoscopy), would you prefer the urinary test or cystoscopy?

Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

6) If the urinary test detects will miss 5 of 100 bladder cancers (vs miss 2 of every 100 cancers in cystoscopy), would you prefer the urinary test or cystoscopy?

Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

PATIENT INITIALS			PATIENT DOB	D	D	M	M	Y	Y

7) If the urinary test detects will miss 4 of 100 bladder cancers (vs miss 2 of every 100 cancers in cystoscopy), would you prefer the urinary test or cystoscopy?		
Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

8) If the urinary test detects will miss 3 of 100 bladder cancers (vs miss 2 of every 100 cancers in cystoscopy), would you prefer the urinary test or cystoscopy?		
Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

9) If a urine test is as good as cystoscopy (miss 2 of every 100 cancers) would you prefer a urinary test or cystoscopy to determine to presence of bladder cancer?		
Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

DETECT II

PATIENT INITIALS			PATIENT DOB	D	D	M	M	Y	Y

10) Would you prefer a urinary test interspaced with cystoscopy to delay the interval between each cystoscopy (ie: 6 monthly cystoscopy with a urinary test at 3 months instead of 3 monthly cystoscopy)?		
Prefer cystoscopy	Neutral	Prefer urinary test
1 ☐	2 ☐	3 ☐

11) Would you prefer if a proposed urinary test is performed by your GP compared to a urinary test performed in hospital	
Prefer Hospital ☐	Prefer GP ☐

12) Why would you prefer a urinary test or cystoscopy? (You may select more than one but select either A or B)			
A) Prefer cystoscopy	☐	B) Prefer urinary test	☐
I would feel anxious if I did not have a cystoscopy	☐	I experience pain/ burning feeling when urinating after cystoscopy	☐
I do not experience side effects after cystoscopy	☐	I experience blood in urine after cystoscopy	☐
Symptoms I experience after cystoscopy do not bother me	☐	To avoid the risk of infection after cystoscopy	☐
I have greater confidence in cystoscopy	☐	I have greater confidence in the urine test	☐
I prefer coming into the hospital for diagnostic tests	☐	I experience no side effects after performing urinary test	☐
The presence of a clinician makes me feel reassured	☐	Having cystoscopy makes me feel anxious	☐
The delay in receiving the results after urinary test bothers me	☐	Urinary test does not require a hospital visit	☐
Any other reason you prefer cystoscopy, please state _____		Any other reason you prefer urine test, please state _____	

DETECT II

PATIENT INITIALS			PATIENT DOB	D	D	M	M	Y	Y

The Brief Illness Perception Questionnaire

For the following questions, please circle the number that best corresponds to your views:

How much does your illness affect your life?

0 1 2 3 4 5 6 7 8 9 10

0 = no affect at all; 10 = severely affects my life

How long do you think your illness will continue?

0 1 2 3 4 5 6 7 8 9 10

0 = a very short time; 10 = forever

How much control do you feel you have over your illness?

0 1 2 3 4 5 6 7 8 9 10

0 = absolutely no control; 10 = extreme amount of control

How much do you think your treatment can help your illness?

0 1 2 3 4 5 6 7 8 9 10

0 = not at all; 10 = extremely helpful

How much do you experience symptoms from your illness?

0 1 2 3 4 5 6 7 8 9 10

0 = no symptoms at all; 10 = many severe symptoms

How concerned are you about your illness?

0 1 2 3 4 5 6 7 8 9 10

0 = not at all concerned; 10 = extremely concerned

How well do you feel you understand your illness?

0 1 2 3 4 5 6 7 8 9 10

0 = don't understand at all; 10 = understand very clearly

How much does your illness affect you emotionally? (e.g. does it make you angry, scared, upset or depressed?

0 1 2 3 4 5 6 7 8 9 10

0 = not at all affected emotionally; 10 = extremely affected emotionally

Please list in rank-order the three most important factors that you believe caused <u>your</u> illness. ***The most important causes for me:-***

1. _____
2. _____
3. _____

DETECT II

PATIENT INITIALS			PATIENT DOB	D	D	M	M	Y	Y

13) Any comments on the questionnaire, cystoscopy of urinary test:

Many thanks for completing this questionnaire.

Kindly enclose and return it using the provided prepaid envelope to:

DETECT II Trial Team

Division of Surgery & International Science,

University College London ,

Room 437,

74 Huntley Street,

London WC1E 6AU

A16: Patient Interview outline

You have been invited to take part in this study as part of the DETECT II study. As part of this study, you have been having a cystoscopy which is the standard investigation to monitor your bladder and detect any recurrence of cancer. You are also providing a urine sample for a new test which may also be able to detect recurrence of cancer.

The aim of this interview is to explore your experience of being diagnosed with bladder cancer and having a cystoscopy and the urinary test as methods of monitoring for cancer recurrence.

I would like to begin by asking questions about your condition

Accessing knowledge of bladder cancer

1) What is your understanding about your cancer? (causes, treatment effectiveness, ways to manage it etc.)

2) How long do you think will your condition last?

Wellbeing

3) How does your illness affect your wellbeing? (well-being, activities of daily living, social roles, work, the use of healthcare services, experiencing symptoms or side effects of treatment)

Assessing experience of cystoscopy for bladder cancer

4) What do you think is the best way to monitor your cancer

5) What do you think about cystoscopy as a method of bladder cancer monitoring?

6) How did you find the experience of having to have cystoscopy?

7) How frequently do you think you will be having cystoscopies from now on?

8) How does it make you feel?

9) How accurate to you think cystoscopy is in terms of detecting cancer?

Attending cystoscopy appointments

10) How do you think you will find attending these appointments at the proposed time-intervals?

Assessing experience using the urine collection kit

11) what do you think about the urine test?

12) What do you think about the urine test you had to do as part of the trial?

13) How did you find providing the urine sample and mailing it back (easy or difficult)?

Access confidence in using urine test for bladder cancer

14) How does the urine test compare with having a cystoscopy?

15) How good would a urine test need to be before you would be happy to accept it instead of cystoscopy?

16) How would you feel about the test as being a standard monitoring method for detecting cancer recurrence instead of cystoscopy?

If NO, please explain why. How accurate do you think the urinary test would need to be in detecting cancer before you would accept it?

17) Consider abbreviated standard gamble

If the urinary test detects will miss (X of 100 bladder cancers (vs misses 2 of every 100 cancers in cystoscopy), would you prefer the urinary test or cystoscopy?

18) If the urine test had similar accuracy to cystoscopy in terms of ability to spot bladder cancer, would you agree to replace all your cystoscopies with the urine test?

Assessing opinion of urine a urine test to reduce the frequency of cystoscopy

19) What do you think about urine the urine test to increase the interval between cystoscopies. Ie:

If YES - how often would you like to have urinary test between your cystoscopies? why at these particular intervals?

If NO, please explain why? How accurate do you think the urinary test would need to be in detecting cancer before you would accept it?

20) Consider abbreviated standard gamble

21) What is your view of using both urine test and cystoscopy to check for bladder cancer recurrence?

22) If it was up to you how often would you like to have a cystoscopy?