THE PREDICTIVE VALUE OF THE NMP22 BLADDERCHEK TEST FOR BLADDER CARCINOMA IN PATIENTS PRESENTING WITH HAEMATURIA TO A SOUTH AFRICAN TERTIARY CARE CENTRE

Mark Richard Purdy

A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree

of

Master of Medicine in the branch of Urology

Johannesburg, 2014

DECLARATION

I Mark Richard Purdy declare that this research report is my own work. It is being submitted for the degree of Master of Medicine in the branch of Urology, in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at this or any other University.

Signature:

M R Purdy

Date: …….. day of ……..…, 2014

Place: Johannesburg

This work is dedicated to my girls. To my loving wife, Candice, for her support, understanding and encouragement. May our daughters, Kate and Amy, be inspired to search for their own answers one day.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS STUDY

Presented as a study proposal at the Biennial Urology Registrars' symposium, Drakensburg, 15 April 2011.

Presented at the University of Witwatersrand's Urology departmental meeting, Johannesburg, 27 August 2013

Presented at the Bert Myburgh Research Forum, University of Witwatersrand, Johannesburg, 27 November 2013

ABSTRACT

THE PREDICTIVE VALUE OF THE NMP22 BLADDERCHEK TEST FOR BLADDER CARCINOMA IN PATIENTS PRESENTING WITH HAEMATURIA TO A SOUTH AFRICAN TERTIARY CARE CENTRE

Bladder cancer is the second commonest urological malignancy and haematuria is the commonest symptom. Cystoscopy and urine cytology are integral for the investigation of haematuria, while the role of molecular markers such as the NMP22 BladderChek test is still being defined. The BladderChek is a qualitative point of care test developed for the detection of the elevated urinary levels of NMP22 associated with bladder cancer. No studies have been performed in South Africa using the BladderChek nor considered using this test to increase the efficiency of the workup of patients with gross haematuria. The primary aim was to establish the percentage of office cystoscopies done as part of a gross haematuria workup at Charlotte Maxeke Johannesburg Academic Hospital that are unnecessary and may be avoided if the BladderChek is positive under defined conditions. A cross-sectional study of the BladderChek test using prospective consecutive sampling, with special care to limit false positives and negatives, of 64 patients with a history of gross haematuria was conducted. The sensitivity, specificity, positive predictive value and negative predictive value for the BladderChek and the urine cytology were 78.9%, 84.4%, 68.2%, 90.5% and 36.8%, 93.0%, 70.0%, 76.9% respectively. The performance of the BladderChek was not affected by the history of gross haematuria, the stage nor grade of malignancy. Urine cytology detected only one malignancy missed by the BladderChek. Approximately 12.6% of office cystoscopies may be avoided and 78.9% of bladder tumours detected if the BladderChek is selectively applied as in this study. This may “fast-track” patients for transurethral resection of bladder tumour. The BladderChek may be a cost-effective alternative to urine cytology.

ACKNOWLEDGMENTS

I would like to thank my supervisor, Professor Mohamed Haffejee for his assistance and support in the preparation of this research report. To Dr Bradley Wood, thank you for giving such practical advice in choosing a research topic and study design. I would also like to thank Dr Gregory Basiewicz for reviewing an early draft of the study protocol and giving valuable advice. Thank you Matritech/Alere for donating the test kits for the study. Thank you to all the urology registrars who accommodated my request to "be down at scopes" every week as the data collection would otherwise have never been possible. In addition, I would like to thank Professor Geoffrey Candy and Dr Esnat Chirwa for their assistance with statistical analysis. Thank you to Mrs Patti Kay and Dr Wendy-Ann Wood for providing insightful advice on the first draft of this research report.

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LIST OF ABBREVIATIONS

AUA: American association of urology

BCa: Bladder Cancer

BCG: Bacillus Calmette-Guerin

BladderChek: NMP22 BladderChek test

CIS: Carcinoma in situ

CMJAH: Charlotte Maxeke Johannesburg Academic Hospital

CT IVP: Computerised tomography intravenous pyelogram (urogram)

EAU: European Association of Urology

ELISA: Enzyme-linked immunosorbent assay

FDA: United States Food and Drug Administration

FISH: Fluorescent is situ hybridisation

FN: False negative

FP: False positive

GH: Gross Haematuria

HG: High grade

IVP: Intravenous pyelogram

KUB: Kidney Ureter Bladder

LA Cystoscopy: Cystoscopy under local anaesthetic

LG: Low grade

MI: Myocardial infarctions

MRI: Magnetic resonance imaging

NBI: Narrow-band imaging

NHLS: National Health Laboratory Service

NMP: Nuclear matrix protein

NPV: Negative predictive value

NUMA1: Nuclear mitotic apparatus protein 1

OCT: Optical coherence tomography

PCa: Prostate Cancer

PDD: Photodynamic diagnosis

PET: Positron emission tomography

POC: Point of care

PPV: Positive predictive value

PSA: Prostatic specific antigen

RBC: Red blood cell

RCC: Renal cell carcinoma

SCC: Squamous cell carcinoma

TCC: Transitional cell carcinoma

TN: True Negatives

TURBT: Transurethral resection of bladder tumour

TURP: Transurethral resection of the prostate

UC: Urine Cytology

USS: Ultrasound scan

UTI: Urinary tract infection

UTTCC: Upper tract transitional cell carcinoma

WLC: White light cystoscopy

CHAPTER 1

INTRODUCTION

1.1 Introduction

Protein synthesis is a central problem for the whole of biology, and that is in all probability closely related to gene action.

- Francis Crick, 1958

Bladder cancer (BCa) is the fourth most common solid organ malignancy in male patients and the second commonest urological malignancy in western countries (eds Soloway, Carmack & Khoury 2005). Haematuria is the commonest presenting symptom of BCa. White light cystoscopy (WLC) and urine cytology (UC) are the standard investigations for BCa in patients with haematuria. BCa is the most expensive malignancy per patient from diagnosis to death (Svatek, Sagalowsky & Lotan 2006). Annual costs for treating BCa in the United States in 2000 were US$1 billion (Konety, Joyce & Wise 2007). With an average of ten cystoscopies per BCa patient lifetime this procedure contributes significantly to cost (Zwarthoff 2008). WLC is problematic not only due to its invasiveness and cost but also the significant false positives and negatives for BCa (Kriegmair, et al. 1996; Sarosdy, Schellhammer & Bokinsky 2002). The poor sensitivity of UC is widely documented and is also a relatively expensive investigation (Glas, et al. 2003). There is a need for an accurate non-invasive cost-effective test for BCa. Several urine-based molecular markers have been studied but the ideal test has not yet been discovered. The NMP22 BladderChek test (BladderChek) is one of the protein-based tests for BCa. Patients with BCa have elevated levels of NMP22 in the urine and the BladderChek is a qualitative point of care monoclonal antibody test for this antigen. The BladderChek may represent an alternative to UC and an important adjunct for the workup of patients with a history of gross haematuria (GH). The BladderChek has never previously been studied in South Africa and the performance in our setting may differ from international studies due to factors such as different pre-test

probabilities and rates of histological subtypes of BCa. Most previous studies, with the exception of Sharma, et al. (1999), did not optimised the performance of NMP22 testing as they did not exclude all factors that may cause a false positive result.

1.2 Problem Statement

- There is no published research from South Africa evaluating the BladderChek.
- International studies may not be applicable in our setting.
- Inclusion and exclusion criteria in previous studies were not optimal for BladderChek performance.

1.3 Significance Of Study

The management of BCa is expensive. Cystoscopy under local anaesthetic (LA cystoscopy) contributes to this cost. LA cystoscopy is currently considered the standard first-line investigation for detection of BCa in patients with haematuria. Detection of BCa on LA cystoscopy usually mandates a transurethral resection of bladder tumour (TURBT) under general or regional anaesthetic for diagnosis and treatment. This means that the LA cystoscopy for BCa is largely a tool of detection which may determine the need for an additional procedure, rather than a tool for definitive diagnosis or treatment. Besides cost, LA cystoscopy is often an uncomfortable procedure for patients. Thus it would be desirable to have a non-invasive, widely available and cost-effective tool which may demonstrate performance similar to LA cystoscopy and result in the same end-result but at decreased cost and morbidity for patients with BCa presenting with GH. The BladderChek may represent that tool. This study will assess the utility of the BladderChek in patients with a history of GH presenting to a South African tertiary hospital.

1.4 Research Questions

- Can a patient with a positive BladderChek omit LA cystoscopy as part of the GH work-up?
- Should UC be abandoned in favour of the BladderChek for transitional cell carcinoma (TCC) BCa diagnosis?

1.5 Aims Of Study

- The primary aim is to establish the percentage of LA cystoscopies done as part of a GH workup at a South African Tertiary Care centre that are unnecessary and may be avoided if the BladderChek is positive under defined conditions.
- The secondary aims in the patients presenting for LA cystoscopy as part of a GH workup are:
  - To compare the predictive value of the BladderChek to UC across various grades and stages and histological subtypes and determine if the BladderChek may be a viable alternative to UC.
  - To compare the demographics, risk factors, symptoms, imaging, BladderChek and UC findings of patients with versus without BCa.

1.6 Study Objectives

- Establish the demographics, risk factors, symptoms, imaging results, BladderChek, UC, cystoscopy and histology findings for patients presenting with a history of GH to a South African tertiary hospital.
- Calculate the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the BladderChek test for BCa in the same group of patients.
- Calculate the sensitivity, specificity, PPV and NPV of UC for BCa in the same group of patients.

CHAPTER 2

LITERATURE REVIEW

2.1 Background To BladderChek Testing

2.1.1 Pathology of bladder carcinoma

The two major histological subtypes of BCa are Transitional Cell Carcinoma (TCC) and Squamous Cell Carcinoma (SCC). TCC is by far the more predominant form in the developed world and our experience in Johannesburg is similar to this and to that reported in the Western Cape (Aucamp & Heyns 1995), although other areas of South Africa serving bilharzia-endemic areas have noted less predominance of TCC with a rate of SCC even up to 25% (Groeneveld, Marszalek & Heyns 1996). There are two distinct clinical forms of TCC, namely the high grade (HG) form and the low grade (LG) form – they are genetically distinct although occasionally both may occur in same patient (Netto 2011). The high grade form is more likely to invade the submucosal tissues including the detrusor muscle (that is muscle-invasive) while low grade is more often non-invasive (Netto 2011). Clearly, the HG invasive form is much more likely to progress and metastasize and early diagnosis of this form is especially desirable. Carcinoma in situ (CIS) is a superficially spreading HG tumour with high risk for recurrence and progression (eds Soloway, Carmack & Khoury 2005). The histologic subtype, grade and stage may all be important variables when using the BladderChek which is discussed further below (2.1.5.3 Current role of the BladderChek test). Less than 2% of all bladder cancers are adenocarcinomas and less than 1% are small cell carcinomas. Even rarer are non-epithelial subtypes of bladder cancer such as sarcoma, lymphoma and phaeochromocytoma (eds Soloway, Carmack & Khoury 2005).

2.1.2 Risk factors for bladder carcinoma

Besides age and sex, BCa also has a strong predominance in the white population (Scélo & Brennan 2007). The most important aetiologic risk factor for BCa is cigarette smoking –

relative risk is 1.5-3.0 compared to non-smokers (Scélo & Brennan 2007). Approximately 30-50% of all BCa is caused by cigarette smoking with a latency period up to 20-30 years (eds Soloway, Carmack & Khoury 2005). The second most common risk factor is occupational exposure although it is often difficult to quantify in the individual patient. Workers in the dye and rubber manufacture industries are considered the highest risk while an increased risk has also been reported in painters, leather workers, shoe makers, aluminium, iron and steelworkers as well as in workers in a number of industries with diesel exhaust exposure (eds Soloway, Carmack & Khoury 2005). Other important risk factors include bilharzia (with relative risk of two to four times and also a long latency period), pelvic radiotherapy and cyclophosphamide (Hirao, Kim & Fujimoto 2009). The relevance of risk factors is that they increase the pre-test probability of the condition in question. This in turn affects the performance of a diagnostic test. As a result, they are important in selecting patients for testing (see 2.1.5.6 Optimising the performance of the NMP22 tests)

2.1.3 Symptoms of bladder carcinoma

The commonest presenting symptom of BCa is haematuria (microscopic or gross), occurring in 85% of patients (Alvarez & Lokeshwar 2007). The prevalence of BCa in males over the age of 60 years with asymptomatic dipsticks haematuria was only 0.5% in one study (Britton, et al. 1992). Similarly, in another study, only 1.3% of 1,034 patients with asymptomatic microscopic haematuria had BCa, although there was also a strong male predominance with almost all BCa patients being over the age of 50 years (Murakami, et al. 1990). In a meta-analysis, the PPV of *gross* haematuria however for urological malignancy was 41% in patients over the age of 40 years with more than 80% of the malignancies being BCa (Buntinx & Wauters 1997). The second commonest symptom of BCa is dysuria especially associated with bladder CIS or muscle invasive disease (eds Soloway, Carmack & Khoury 2005). Thus gross haematuria is an important predictor of BCa especially in male patients and especially with advancing age. Once again, by careful patient selection, one may increase the pre-test probability of BCa (see 2.1.5.6 Optimising the performance of the NMP22 tests).

2.1.4 Workup of haematuria

The current approach to a patient presenting with haematuria to the University of the Witwatersrand Academic Hospitals (haematuria workup) is to take a history, including assessment of risk factors for BCa, and do an examination. Urine is sent for UC and urinalysis (microscopy and culture) and blood tests, including a prostatic specific antigen (PSA) in male patients, are performed. The upper tracts are investigated preferably by computerised tomography intravenous pyelogram (CT IVP), or by means of renal ultrasound scan (USS) together with an IVP. Regardless of the results of all of the above though, and often before this is even done due to prolonged waiting times for radiologic investigations, patients generally undergo a LA cystoscopy using a rigid cystoscope on a different day to assess (mainly) for any bladder tumour. In principle, this approach is consistent with the current European Association of Urology (EAU) guidelines although usually flexible cystoscope under local anaesthetic is done in the rooms – so called "office cystoscopy" (Babjuk, et al. 2013). Imaging (CT/MRI/PET) of bladder tumours is mainly for staging of the primary and assessment for lymphatic and visceral metastases rather than the initial detection.

2.1.4.1 White-light cystoscopy

WLC alone cannot be considered the gold-standard for the diagnosis of BCa due to:

- False Negatives (FN): WLC has been shown to have a significant FN rate with a sensitivity even as low as 73% (Kriegmair, et al. 1996). The sensitivity for CIS is even lower.
- False Positives (FP): Another multicentre study noted positive histology in only 37% of WLC performed by experienced urologists where the WLC was considered suspicious and a biopsy was performed (Sarosdy, Schellhammer & Bokinsky 2002).

This highlights the need for better tools in the diagnosis of BCa. The current potential adjuncts include bladder mapping biopsies, advanced endoscopic techniques, urine cytology and molecular markers.

2.1.4.2 Bladder mapping biopsies

Bladder mapping biopsies are currently considered controversial. It is widely accepted that bladder CIS is associated with an increased risk of recurrence and progression and WLC has a poor sensitivity for detection of CIS. One recent study compared WLC alone versus WLC combined with bladder mapping biopsies but the latter still reported a sensitivity for CIS of only 46% using pathological examination of cystectomy specimen as the gold standard (Gudjonsson, Blackberg & Chebil 2012). Thus bladder mapping biopsies may improve the sensitivity of WLC for CIS detection but the sensitivity is still poor. In addition, a recent meta-analysis of WLC, photodynamic diagnosis (PDD) and mapping biopsies, reported that in the largest study analysed, just 12% of patients with CIS were detected on random biopsies but missed on WLC and PDD (Isfoss 2011). Routine bladder mapping biopsies are not advocated by the American Urological Association (AUA) nor the EAU guidelines (Hall, et al. 2007; Babjuk, et al. 2013). The current EAU guidelines and the International Consultation on Urological Diseases (ICUD) recommend bladder mapping biopsies only if the UC is positive with a normal cystoscopy and upper tract assessment, or if a partial cystectomy is planned (Kamat, et al. 2013).

2.1.4.3 Advanced endoscopic techniques

The most well studied advanced endoscopic technique currently is photodynamic diagnosis (PDD). PDD, also known as fluorescent-light cystoscopy, is the use of blue-spectrum light for cystoscopy after intravesical administration of photoactive porphyrins (hexaminolevulinic acid or 5-aminolevulinic acid) which preferentially accumulate in neoplastic cells and result in a red fluorescence. The ideal gold standard for calculating the sensitivity of PDD would be pathological examination of the cystectomy specimen, but current studies consider WLC together with PDD as the gold standard. PDD is significantly better than WLC for detection of CIS. In a recent meta-analysis on PDD, the sensitivity for CIS of PDD was 92.5% versus 60.5% for WLC where the gold standard was considered as the total number of CIS lesions detected on WLC and PDD together (Isfoss 2011). Narrow-band imaging (NBI) is a technology which uses a filter to modify the endoscopes light to create a narrow bandwidth which is centred on the haemoglobin's absorption band (Emura, Saito & Ikematsu 2008). Tumours have an increased vascularity thus this modification optimises visualisation of them.

The low specificity (60-85%) is one problem of NBI (Kamat, Hegarty & Gee 2013). Large trials are still needed to validate this technology. Optical Coherence Tomography (OCT) is a technology which has been most well studied in ophthalmology. In principle, the technique of generating an image with OCT shares many similarities to ultrasound scanning. It has recently been studied as an adjunct to cystoscopy. Preliminary small nonrandomised studies of OCT are promising with sensitivity and specificity of 92-95% (Kamat, Hegarty & Gee 2013). Thus PDD is the most well studied advanced endoscopic technique, it is more sensitive than WLC especially for CIS and is considered by some as the new gold standard for BCa diagnosis when combined with WLC.

2.1.4.4 Urine cytology

UC has long been considered the standard adjunct to WLC. In most studies, the specificity of UC is high but the sensitivity is poor, especially for low-grade, low stage tumours (Lokeshwar & Soloway 2001). In the largest study of WLC, BladderChek and UC, the BladderChek test detected 32 BCa missed by UC, while UC was positive in only two BCa missed by the assay (Grossman, et al. 2005). Factors affecting the sensitivity include the specimen quality (for example voiding versus washings specimen), number of exfoliated cells, and pathologist expertise (Talwar, et al. 2007). Halling, et al (2000) noted that the sensitivity of UC was significantly less after 1990 compared with before 1990. The authors suggested that a potential explanation was the loss of the expert skill of UC which is being done more by general pathologists in recent times. Similarly, a systematic review from 2003, noted that the sensitivity of UC decreased linearly from 80% to 52% between 1990 and 2000 (Glas, et al. 2003). A common misconception by clinicians is that UC is a simple matter of positive or negative. It is unfortunately much more a subjective interpretation by the cytopathologist with a 15% inter-observer variation reported despite a uniform nomenclature system. “Pathologists do not “read” slides, as if all of the information were clearly written in some arcane text…rather, they function more as critics, who are given a single snapshot of a game in progress and expected to divine the game and its likely outcome” (eds Soloway, Carmack & Khoury 2005). Accuracy may be aided by additional clinical information such as voided versus washings specimen, history of prior intravesical therapies, and history of prior high grade TCC but the interpretation is often performed without this additional clinical information. This commonly results in the ambiguous reports of atypical cells or suspicious

cells and possibly accounts for the widely variable sensitivities reported across studies. Besides the need for a trained cytopathologist, UC is also more expensive and the result takes longer than the BladderChek. Due to its poor sensitivity, especially in recent studies, some centres have even abandoned UC altogether (Sangar, Ramani & George 2008). In fact, one may suspect that almost all urologists outside academic centres in United States have abandoned the test with only 3.3% of BCa patients having UC performed in 2001 (Konety, Joyce & Wise 2007). This is unfortunate since most experts still consider it an important test for diagnosis of CIS which may not be appreciated by community urologists. In addition, the cytopathologist's skill may be lacking outside academic centres and so the benefit may be further diminished.

2.1.4.5 Tumour markers

Although the advanced endoscopic techniques discussed above are accurate, they are invasive and expensive hence the desire for simpler, cheaper, non-invasive options for BCa detection and surveillance. Conversely, UC is a non-invasive option but is problematic mainly due to its poor sensitivity. Several tumour markers that are present in the urine have been identified which may have clinical utility for BCa in terms of screening, diagnosis, prognosis (risk of recurrence) or surveillance (Nguyen & Jones 2008). Besides the BladderChek, the following are the most well studied BCa tumour markers that are currently approved by the United States Food and Drug Administration (FDA) and are available in current clinical practice (Kamat, et al. 2013):

- Bard Tumor Antigen (BTA) test: This is an immunoassay for detecting human complement factor H related protein (CFHrp). It is postulated that this protein may give cancer cells selective growth advantage by escaping complement-mediated cell lysis (Kinders et al. 1998). The CFHrp assays are available as a qualitative point of care test (BTA Stat) and a quantitative reference laboratory test (BTA Track). The technique for performance and interpretation of BTA Stat is very similar to the BladderChek. Importantly, although still called the BTA test, the current antigen used for testing by Bard is different to the original bladder tumour antigen which was a basement membrane protein released when the tumour invaded the stroma – this means the current test (since 1996) is different to the original and thus earlier reports of sensitivity and specificity of

the original BTA test should not be extrapolated to the current BTA test (Konety & Getzenberg 2001). Generally the BTA test is more sensitive than UC. Most studies reported a lower sensitivity, a higher false positive (FP) rate and lower PPV using the BTA compared with the BladderChek tests (Ross & Cohen 2001). Causes of FP BTA tests are similar to the BladderChek but in addition include haematuria itself since complement factor H is present in normal human serum (Lokeshwar & Soloway 2001).

- Immunocyt test: This test combines UC with immunofluorescence to improve the sensitivity of UC alone for detecting low-grade tumours but with loss of specificity compared to UC alone. One study reported an overall sensitivity of 46.8% and 86.1% and specificity of 98.2% and 79.4% for UC and Immunocyt respectively (Mian, et al. 1999). Of note, also in this study of the 264 patients with symptoms or a prior history of TCC BCa, the sensitivity for Grade 1 TCC was 4% and 84% for UC and Immunocyt respectively. It is only approved by the FDA for surveillance in conjunction with UC (Budman, Kassouf & Steinberg 2008). It requires specialised equipment and cytological knowledge and expertise. The cost is a major prohibitive factor.
- UroVysion test: This test uses fluorescent in situ hybridization (FISH) to detect increased number of copies of chromosomes 3, 7, 17 and homozygous deletions at the 9p21 locus. High sensitivity (81%) and specificity (96%) for HG tumours is reported (Konety & Lotan 2008). Cost is prohibitive for routine use but may ultimately decrease with automation (Konety & Lotan 2008). In a multicentre, prospective, blinded comparative trial, 40% of patients with positive FISH assay and negative cystoscopy had visually evident tumour at mean of six months but even up to sixteen months later. The authors coined the term “anticipatory positive” for this phenomenon. Conversely, in patients with a negative FISH and a negative cystoscopy, only 20% of patients at mean of 11 months had visible tumour (Sarosdy, et al. 2002). Due to the “anticipatory positive” phenomenon and the very high specificity of this test, it has been recommended that patients with a positive UroVysion and an otherwise negative workup be followed up closely and bladder mapping biopsies even be considered (Chiong, Gaston & Grossman 2008).
- BladderChek: This is available as both a quantitative laboratory assay and a point of care device. It is discussed in detail below (2.1.5).
- Tumour marker panel: It has been suggested that a panel of tumour markers may be ideal to maximise the sensitivity and specificity of the various tests. Cost is a concern and the ideal panel is yet to be decided (Parekattil, Fisher & Kogan 2003).

Thus the BTA stat is most similar to the BladderChek in terms of being an affordable point of care (POC) test but most studies have reported that the BladderChek is more accurate. Conversely, the Immunocyt and in particular the UroVysion test are more accurate than the Bladderchek but their cost makes them impractical for routine use.

2.2 BladderChek

2.2.1 NMP22

NMP22 is a nuclear matrix protein (NMP) also known as nuclear mitotic apparatus protein 1 (NUMA1). NMP's provide a framework to structurally and functionally organize DNA within the nucleus. NMP22 is important for spindle stabilization during mitosis. It is present in all epithelial cells, glandular cells, cells in lymph nodes and macrophages but the bladder epithelium expresses NMP22 at an especially high level (Miyake, et al. 2012). The reason for the elevated levels of NMP22 in the urine of patients with BCa is controversial. Some have suggested that NMP22 is at least 25 times more prevalent in malignant than normal urothelial cells (Keesee, et al., 1996 *in* Poulakis et al. 2001). Recently this has been disputed with BCa tissue actually staining *less* intensely for NMP22 than normal urothelium and it has been suggested that elevated urinary levels of NMP22 are simply due to the high rate of cell turnover of tumours with lysis of shedded cells in the urine (Miyake, et al. 2012). NP22 levels are higher in larger tumours and in tumours of higher grade or stage (Shariat, et al. 2004). Use of a morning urine sample (before noon) has been reported as more reliable than an afternoon sample but the exact reason for this phenomenon is unknown (Chen, et al. 1997).

2.2.2 The BladderChek test

The original NMP22 test was a quantitative Enzyme-linked immunosorbent assay (ELISA) performed in a reference laboratory. The BladderChek is a lateral flow

immunochromatographic qualitative POC assay which uses monoclonal antibodies to detect the NMP22. It uses 4 drops of freshly-voided urine (preferably maintained in the bladder for at least two hours before collection) and gives a result after 30 minutes. In the test zone, the NMP22 antigen reacts with an antibody forming a complex which forms a visible line if the NMP22 concentration is more than 10 U/ml while a control line indicates that the device is working properly. Any test line, however small and faint is judged as positive. The value of 10 U/ml was initially suggested based upon maximal sensitivity and specificity in the landmark study by Soloway, et al (1996) and subsequently confirmed by others. The optimal cut-point however is controversial. One prominent multicenter study suggests that the cut-point should be 6.5 (Shariat, et al. 2004). The author's argument however for a lower cut-point is weak because as expected their data clearly shows that one simply increases sensitivity but decreases specificity with a lower cut-point. The result is an almost identical accuracy and an exactly identical balanced accuracy for detecting cancer overall. The difference in opinion regarding the cut-point is likely linked to the mathematical influence of the prevalence of BCa in the study population on the accuracy calculation. The high prevalence (52%) of BCa in the study by Shariat, et al. (2004) likely influenced their choice of lower cut-point as sensitivity would then influence the calculated accuracy more while in the study by Soloway, et al (1996) the lower prevalence (42%) of BCa would mean specificity would have the greater influence on the calculated accuracy and thus the higher cut-point would be a better choice. As the prevalence of BCa in patients evaluated for possible BCa is usually less than 50% the higher cut-point would seem to be the better choice to maximize the accuracy in clinical practice. Fortunately, the cut-point of 10U/ml was chosen for the BladderChek test and is also the most widely used cut-point for the ELISA test. The BladderChek is the only POC test approved by the FDA for *both* detection and surveillance of BCa (Budman, Kassouf & Steinberg 2008).

2.2.3 The current role of the BladderChek

One of the most important advantages of urine-based tests such as the BladderChek is that it avoids patient morbidity as it is painless and non-invasive. One study reported a third of patients found flexible cystoscopy under local anaesthetic to be "quite" painful or discomforting (Van der Aa, et al. 2008). The BladderChek in particular is easy to use and does not require the expertise of a cytopathologist. The result of this POC test is available

during the patient's consultation (within 30 minutes) which may certainly be a benefit if one considers the result of a recent study which found that 19% of patients reported the delay waiting for laboratory urinary results at least as quite discomforting or burdensome (Van der Aa, et al. 2008). Compared to other FDA-approved urinary molecular marker tests, the BladderChek is also cost-effective as the UroVysion and ImmunoCyt cost more than ten times the BladderChek or the BTAstat test.

The role of the BladderChek test, like all other available urinary molecular marker tests, is still being defined. Alvarez and Lokeshwar (2007) point out that the urinary molecular markers for BCa are actually better tests than the PSA is for prostate cancer detection, yet none are widely used for BCa detection perhaps due to urologists' unfamiliarity and unrealistic expectations of a "perfect marker" for every setting in BCa. The ICUD does not currently recommend any urinary molecular markers for BCa screening or follow-up due to lack of high level evidence. In addition, they state that "It is actually unlikely that an "ideal" marker will be sufficiently accurate to help in the different diagnostic scenarios (i.e. screening, early detection, and surveillance for low- and high-risk disease)." (Kamat, et al. 2013). The following explores the potential roles of the BladderChek further:

- Diagnostic adjunct:

The main diagnostic use of the BladderChek currently is in the patient with a normal cystoscopy. If the BladderChek is positive then this suggests a possible missed tumour on cystoscopy, occult bladder tumour (CIS) or upper tract TCC (UTTCC). In the largest study of the BladderChek test, the combination of the BladderChek and cystoscopy detected 93.7% of malignancies vs 88.6% for the initial cystoscopy alone. In addition, no patients had a positive UC with a negative BladderChek and a negative cystoscopy – suggesting that UC is superfluous to other two tests (Grossman, et al. 2005). Unfortunately, like UC, the sensitivity and specificity of BladderChek is generally worse for smaller, low grade and stage tumours (Boman, Hedelin & Holmang 2002). An additional diagnostic concern is the high FP rate in certain patient groups. Patients with urinary tract infections (UTI's), urinary stones or foreign bodies, intravesical Bacillus Calmette-Guerin (BCG) within the preceding three months, recent urinary tract manipulation or bowel interposition within urinary tract may all

experience a FP BladderChek result. Correction of the NMP22 level for urine concentration using the urinary creatinine level has been examined but the accuracy was found to be not significantly different and probably irrelevant in a once off measurement (Sanchez-Carbayo, et al. 1999). In a subsequent study by the same lead author, it was noted that correction for urinary concentration may be important in individual patients on surveillance whose own NMP22 level at baseline serves as a control. This practice increases sensitivity but decreases the specificity and PPV of the test (Sanchez-Carbayo, et al. 2001). The practice of correction of NMP22 level for urine concentration has not been widely adopted and is clearly not possible with the BladderChek test. Recently, a NMP22-based nomogram has been described which includes age, gender, smoking status, race, degree of haematuria and the BladderChek result. On external validation of the nomogram, the reported accuracy of the BladderChek was 76% and of the nomogram was 82.4% with virtually no additional added value of UC (Lotan, et al. 2009).

- Surveillance

Molecular markers may be useful for two aspects of surveillance:

a. Risk Stratification to determine the surveillance protocol: Although the data is conflicting, the BladderChek seems to predict the risk for recurrence but not progression. Thus low and intermediate-risk BCa patients with a negative BladderChek may be candidates for less intense follow-up (Gupta, Sharma & Kumar 2009). Conversely, a nomogram has been developed based upon more than 2500 patients across 10 centres and may predict probability of recurrence, and probability that the recurrence is high grade or muscle invasive. The accuracy varied across institutions (Shariat, et al. 2005).

b. Surveillance technique itself: Many studies have reported that the overall sensitivity of the BladderChek is superior to UC. One study reported sensitivities of 67.3% versus 21.1% and the authors suggested that UC "can be omitted in favor of NMP22 in follow-up of low-grade superficial bladder TCC" (Talwar, et al. 2007). However, the reported sensitivity is highly variable for *recurrent* tumours. One group reported a sensitivity of only 45% despite the low NMP22 cut-point of only 4u/ml which the authors attributed to the small size of recurrent tumours (Boman, Hedelin & Holmang 2002) while another group counter-intuitively reported both a better sensitivity (85%) together with a better specificity for recurrent tumours using the BladderChek which has a higher cut-point of 10u/ml inherent to it's design (Gupta,

Sharma & Kumar 2009). The reasons for this discrepancy is unknown but the BladderChek test may be performing better than the ELISA test due to freshness of the specimen or may be detecting another unknown antigen. Nevertheless, the sensitivity is still not high enough to omit surveillance cystoscopy altogether but there is ongoing research assessing if it is possible to safely substitute alternate surveillance cystoscopies for NMP22 testing instead.

- Screening

This is problematic using the BladderChek due to the poor sensitivity and FP's in an unselected population. It may be appropriate and cost-effective in high-risk target populations (Svatek, Sagalowsky & Lotan 2006). The NMP22 level is a continuous variable with higher levels associated with increased grade and stage. As such the choice of the cut-point depends upon the goals of screening. Normal healthy volunteers have NMP22 levels less than 3.0 (Shelfo & Soloway 1997). If the goal is to detect the majority of BCa then one may select a lower cut-point such as 6.5U/ml but if goal is to detect the majority of potentially life-threatening aggressive BCa then may select higher cut-point such as 10U/ml (Shariat, et al. 2004). One may optimize the sensitivity at the cost of the specificity with particular combinations of markers but the best combination is still to be defined and is limited by cost (Horstmann, et al. 2009).

- Prognostic

NMP22 levels may have prognostic value since they directly correlate with tumour size, grade and stage, risk of recurrence and progression (Shariat, et al. 2004; Nguyen & Jones 2008).

- Adjunct to haematuria workup

This is the crux of this research report. Sharma, et al (1999) made the following statement in the discussion of their landmark paper almost as an afterthought: "Another role of NMP22 in patients with recurrent disease may be to schedule sedative procedures for those with positive NMP22 values, when we expect a necessary biopsy, and to reserve flexible cystoscopy for those with negative values". Besides this statement, I am unaware of anyone that has explored this idea further. The effect of haematuria on the NMP22 tests' accuracy is controversial. Atsu et al (2002) reported that haematuria affected the diagnostic accuracy of the NMP22 ELISA test. There is no clear biologic reason for this since red blood cells (RBC)

do not have a nucleus and thus no nuclear matrix proteins but these authors suggest that this observation may be due to the RBC membrane proteins or some unknown serum proteins (Atsu, et al. 2002). Similarly, another group recently managed to generate a FP on the BladderChek test when adding 50 microlitres of whole blood per 10 millilitres of urine but not when adding serum suggesting that the cause of the FP is due to a cellular component in blood (Miyake, et al. 2012). In contradiction, another study concluded that the effect of haematuria on the ELISA test was "low but significant" although their data table actually shows no difference in sensitivity for BCa in patients with or without gross haematuria (Boman, et al 2002). Further, a recent study also reported that haematuria, even if gross, had no significant effect on the FP rate of the BladderChek test unlike all other POC BCa molecular tests evaluated. In this study, an experimental model was created by spiking urine with various concentrations of blood (Hennenlotter, et al. 2011). Thus it seems that although haematuria may have an effect on the NMP22 tests, this effect is inconsistent between studies and may be less with the BladderChek compared with the NMP22 ELISA test.

2.2.4 Diagnostic accuracy of the BladderChek

2.2.4.1 Reported accuracy of the BladderChek

The sensitivity, specificity, PPV and NPV of NMP22 tests vary between studies and have been shown to be affected by the study population or pre-test probability. Arguably, the three most important reference studies are:

- Sharma, et al. 1999: This study identified six exclusion criteria to obtain a specificity of 95.6% and PPV of 87.5%.
- Lotan & Roehrborn 2003: This meta-analysis reported a sensitivity of 73% and specificity of 80% (Lotan, Roehrborn 2003).
- Grossman, et al. 2005: This is the largest NMP22 study to date and used the BladderChek. It was a multi-institutional study involving 1331 patients, and reported a sensitivity of 55.7% and specificity of 85.7% without the application of exclusion criteria. In the same study, it was shown that a positive BladderChek may even detect bladder tumours initially overlooked on initial cystoscopy. In addition, a negative cystoscopy

together with a negative BladderChek increased the confidence of no underlying bladder tumour from 88.6% to 93.7%. (Grossman, et al. 2005).

2.2.4.2 Accuracy of the BladderChek in South Africa

The BladderChek test has never been previously studied in South Africa. One may speculate that the performance may be better locally as larger and more advanced tumours may be expected due to delayed referrals in the less well developed health care system of South Africa compared with the United States and many parts of Europe where the BladderChek has previously been studied. The NMP22 values have also been reported as significantly higher in bilharzial SCC than in TCC (Eissa, et al. 2002). As there is a higher proportion of bilharzial-associated SCC in South Africa than in the western countries where the majority of NMP22 research has been performed, it would be expected that the sensitivity for BCa would be higher in our patient population although the same factor may also impair the specificity (Hutterer, et al. 2008). The reason for the decreased specificity may be the reported FP's due to bilharzia (Kapila, et al. 2008). Similarly, Grossman (2000) mentions in an editorial comment that NMP22 performance is different depending upon the studied population (Casella, Huber & Blochlinger 2000). All this suggests the need to define the utility of the BladderChek in South Africa.

2.2.5 NMP-22 levels and malignancies other than BCa

There is limited data regarding urinary NMP22 levels in malignancies other than BCa.

- Upper tract transitional cell carcinoma (UTTCC): The BladderChek has shown good sensitivity and specificity for UTTCC with maximal diagnostic accuracy when combined with urine cytology of a voided specimen – sensitivity 79.4% and specificity of 88% in a selected group of patients with UTTCC. Interestingly in this study from the Balkans the authors used 25 patients with known renal stones as the control, yet only two of these patients had a positive BladderChek so they still managed to report a high specificity for UTTCC overall (Jovanovic, et al. 2011).
- Renal Cell Carcinoma (RCC): Three small studies have been reported. The first study included six patients with urinary calculi in the control group, two of whom had elevated

NMP22 levels. Despite this, the average NMP22 levels were significantly higher in RCC group with 40% of RCC patients having values 10 U/ml or above (Huang, et al. 2000). The second study also used patients with calculi in the control group but still reported a sensitivity of 47% and specificity of 90% with NMP22 cutoff of 9.6 (Ozer, et al. 2002). The latest study, again used patients with renal calculi in control group and 60% of RCC patients had NMP22 levels>10U/ml (Kaya, et al. 2005). Thus extrapolating from the published NMP22 ELISA data, RCC may certainly cause BladderChek positivity.

- Prostate Cancer (PCa): Limited data but prostate cancer has been reported as a cause of false positive NMP22 test (Zippe & Pandrangi 1999).

2.2.6 Optimising the performance of the NMP22 tests

2.2.6.1 Pretest probability and Bayes theorem (theorem of conditional probability)

This is a somewhat intuitive concept for clinicians. As additional information is gained from history, examination and special investigations, the probability of certain diagnoses increase while others decrease. The factors on history may include demographic factors such as patient's age, sex, race as well as symptoms. The pretest probability is relevant to any diagnostic test since the higher the pretest probability, the better the performance (accuracy) of the test.

2.2.6.2 Optimising the pretest probability and avoiding FP on the NMP22 tests

- Inclusion criteria

A study from the NMP22 study group (Lotan & Shariat 2008) illustrated how gross haematuria as an inclusion criterion may significantly impact the PPV of the BladderChek test. The PPV of microscopic haematuria was 8.0% for urinary tract cancer in women and 28.6% if gross haematuria. In males, the corresponding figures were 14.6% and 51.2%. In addition, in those males with gross haematuria who also smoked the PPV (that is the chance of an underlying urinary tract malignancy) was more than 70%. Another illustration of the

importance of gross haematuria is in the NMP22-based nomogram where gross haematuria contributes more than any other clinical factor, and even more than the NMP22 result, in predicting the probability of BCa (Lotan, et al. 2009).

- Exclusion criteria

To maximise pre-test probability, patients under 40 years of age may be excluded due to the very low rate of BCa in this age group. Other exclusion criteria may be used to both optimise the pre-test probability and avoid known causes of FP NMP22 test. The specificity of the NMP22 tests may be improved by either increasing the cut-off value, such as to 20 U/ml but at the expense of sensitivity, or by the application of exclusion criteria. Six exclusion criteria which accounted for 80% of FP were applied by Sharma, et al (1999) to increase the specificity and PPV from 82% to 95.6% and 38.9% to 87.5% respectively. The six exclusion criteria were benign inflammatory conditions (cystitis, UTI, prostatitis), urinary calculi, foreign bodies in the urinary tract (stent, nephrostomy), bowel interpositions in urinary tract, other genitourinary cancers (PCa, RCC), and instrumentation of the urinary tract. Unfortunately the authors did not provide details of their criteria for the diagnosis of these conditions. Another study from Cleveland Clinic applied similar exclusion criteria to increase the specificity and PPV from 83.9% to 99.2% and 34.1% to 92.0% respectively without any loss in sensitivity (Ponsky, et al. 2001). Urine dipsticks may be used as a screening tool as a meta-analysis reported the NPV for UTI in urology patients if the dipsticks is negative for nitrite is 93% and is 98% if also negative for leucocytes (Deville, et al. 2004). In addition to the criteria by Sharma, et al (1999), prior intravesical BCG has shown to elevate NMP-22 levels (Lee, et al. 2000). The mechanism for all these factors is increased epithelial cell turnover with the factor having by far the greatest effect being interposition of bowel in the urinary tract (Ishii, et al. 2001). NMP22 values have been shown to be unaffected by urinary tract manipulation more than five days before (Soloway, et al. 1996).

2.2.7 Urinary NMP22 levels and pregnancy

Urinary NMP22 levels have been reported to increase during pregnancy, but even at their peak in the third trimester are usually below 10u/ml so are unlikely to cause a FP on the

BladderChek (Guven, Kilinc & Batukan 2005). Regardless, if one excludes patients less than 40 years of age with the goal of maximising the pre-test probability, then one also excludes the majority of the childbearing population, so this factor is unlikely to be practically important.

2.2.8 Cost analysis of the BladderChek

A detailed cost-analysis is beyond the scope of this report but it is worthwhile putting the cost of the BladderChek into perspective compared with other urinary molecular markers and other investigations that are done as part of a haematuria workup.

2.2.8.1 Comparison of cost of BladderChek with other urinary molecular markers

Table 2.1 gives the current retail cost of the BladderChek in South Africa together with the cost of the other FDA-approved urinary molecular markers for comparison. Immunocyt/ucyt+ is not currently available in South Africa but costs a couple hundred dollars in North America.

Table 2.1 *Cost comparison of urinary molecular markers*

Test	Company	Retail Cost
NMP22 BladderChek	Matritech/Alere	R105 per test kit (R2,520 per box of 24)
BTAstat	Polymedco	R85 per test kit + shipping/duties (US$85 per box of 10)
UroVysion	Abbott*	Approx. R4,000
Immunocyt/ucyt+	Scimedx	Unavailable in RSA
* Performed by NHLS at Charlotte Maxeke Johannesburg Academic Hospital		

2.2.8.2 Cost of other investigations as part of haematuria workup

It is worthwhile putting the cost of the BladderChek test in perspective compared with the cost of the other investigations which are commonly performed during the haematuria workup (Table 2.2). The most important amount to note in this table is the cost of UC whether done in the public or private sector and to compare this with the cost of the urinary molecular markers above. The public sector "urologist" and "radiology" fees below were obtained from the billing manual used in public hospitals to calculate charges for patients classified as "private". The private sector "urologist" fees were the former "medical aid rates" which is usually the bare minimum and which is often considerably more in practice. The laboratory fees for the public and private sectors are the National Health Laboratory Service (NHLS) and Lancet laboratory charges respectively. The private sector radiology fees were obtained from two prominent private hospitals in Johannesburg which probably represent a reasonable spectrum.

Table 2.2 *Cost of haematuria investigations*

	PUBLIC SECTOR	PRIVATE SECTOR
UROLOGIST		
Office Consultation	R241	R250
Cystoscopy	R423	R400
LABORATORY		
Urine m,c&s	R176 (R45 + R58 + R73)	R329 (R57 + R228 + R44)
Urine cytology	R142	R390
FBC	R96	R148
U&E	R204	R227
PSA	R133	R168
RADIOLOGY		
KUB	R79	R190-R462
Abdo USS	R317	R400-R710
IVP	R317	R1,420-R2,300
CT IVP	R1,520	R3,000-R9,080

Urine m,c&s: Urine microscopy, culture & sensitivity; FBC: Full blood count; U&E: Urea, creatinine and electrolytes; PSA: Prostatic specific antigen; KUB: Kidney Ureter Bladder X-ray; Abdo USS: Abdominal ultrasound scan; IVP: Intravenous pyelogram; CT IVP: Computerised tomography intravenous pyelogram

2.3 Summary

In summary, the BladderChek is an affordable and non-invasive POC test. It has shown good accuracy in prior studies including being more accurate than UC. It is possible to optimise the performance of the BladderChek by using various criteria to increase the pre-test probability and exclude known FP's before the test is used. The BladderChek has never previously been studied in South Africa and its accuracy may differ locally for several reasons. Even though the effect of haematuria on the test is controversial, there is sufficient reason to surmise that it may be able to increase the efficiency of the current workup of patients for BCa.

CHAPTER 3

PATIENTS AND METHODS

3.1 Study Design

A cross-sectional study of the BladderChek test using prospective consecutive sampling, with special care to limit FP's and FN's, in patients with a history of gross haematuria was conducted.

3.2 Setting

Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) is a tertiary level hospital with 1,088 inpatient beds. There are approximately 10,000 urology outpatient consultations per year.

3.3 Materials

UriChek™ urine dipsticks manufactured by Omnipharma were used to test for blood, leukocytes and nitrite. BladderChek tests were obtained directly from manufacturer (Alere).

3.4 Participants

Participants were recruited from the CMJAH, when they presented for LA cystoscopy as part of a haematuria work-up.

All patients with gross haematuria were evaluated for inclusion in the study at the time of presentation for cystoscopy on a designated day separate from the time of initial evaluation in the outpatient clinic. The study was explained to all eligible patients who were then invited to participate. All patients who agreed to participate signed a consent form.

Inclusion criteria

- Gross haematuria (current/recent)

Exclusion criteria

- Age <40yrs
- Concurrent UTI defined as positive nitrates on dipsticks or on recent urine m,c&s.
- Concurrent urinary tract foreign body (e.g. stent) or stone visible on KUB x-ray.
- Any urinary tract manipulation within the preceding five days
- Current urinary catheter
- Urinary tract bowel interposition
- Prior pelvic irradiation
- BCG within the preceding three months

3.4.1 Recruitment of at least 100 patients was planned based upon a sample size calculation using Epi Info assuming a confidence level of 95% and precision of at least 10% with a projected test performance of 70% sensitivity and specificity.

3.5 Procedure

3.5.1 A study specific history was performed for all patients presenting for LA cystoscopy with a history of gross haematuria.

3.5.2 By the end of the history, if the participant was disqualified from the study due to any particular exclusion criterion, the reason was clearly explained to them and noted. If not disqualified, then continued as below.

3.5.3 Informed consent was obtained. The study was explained to participants and any questions were addressed. Patients were given an information sheet (Appendix 1). All patients were free to decline participation without being disadvantaged in any way. If they agreed, an informed consent form was signed (Appendix 2). A sequential participant study number was assigned using the patient anonymity sheet which was then used on the data collection sheet (Appendix 3). Names and hospital numbers were recorded only

on the patient anonymity sheet which was kept separately away from the data collection sheets.

3.5.4 An abdominal examination was performed. The study history and examination were in addition to the routine full history and examination that is done for all patients at the urology outpatient clinic or for patients admitted to the urology ward.

3.5.5 Parameters that were recorded specifically for the study:

3.5.5.1 Demographics: Age, Sex and Race

3.5.5.2 Symptoms:

- Haematuria history – gross or microscopic
- Storage symptoms
- Abdominal pain

3.5.5.3 Risk factors:

- Smoking history – current or within the last 20 years
- Occupational history
- Bilharzia history
- Cyclophosphamide history

3.5.6 The KUB x-ray which is usually done routinely for haematuria workup at our institution was reviewed to confirm no concurrent (radio-opaque) urinary stones or foreign bodies. If an USS, IVP, CT or MRI had already been done, this was also reviewed.

3.5.7 In male patients, the PSA was recorded (or if not done, the blood sample was taken before the cystoscopy).

3.5.8 Voided urine was collected into a plastic container, the patient having not voided for at least the preceding 2 hours. The urine sample was divided into three equal aliquots – one was tested immediately using a standard urine dipsticks. If the dipsticks were negative for nitrite then the same aliquot was used for the BladderChek test. The BladderChek test was performed at room temperature using 4 drops of urine and the result was read at between 30-45 minutes. The other two aliquots of urine were

submitted immediately for UC and culture respectively. The personnel conducting the cytological examinations were unaware of the cystoscopy or BladderChek results or even the patient's participation in the study.

3.5.9 LA cystoscopy was done by one of the urology registrars as per the routine functioning of the department. The registrar performing the cystoscopy was blinded to the BladderChek result.

3.5.10 If a tumour was found on LA cystoscopy, either a cold-cup biopsy was done or the patient was scheduled for a TURBT as per the routine functioning of the urology department without knowledge of the BladderChek result.

3.5.11 The bladder biopsies and TURBT specimens were processed for haematoxylin and eosin (H&E) staining by the NHLS. The pathologists were blinded with respect to patient participation so as not to influence grading or staging. Staging was according to TNM system of American Joint Committee on Cancer (AJCC).

3.5.12 Participants were counselled at their next oncology clinic visit regarding the result of the TURBT histology as per routine.

3.5.13 UC result was recorded as positive, atypical or negative.

3.5.14 The pathology database was reviewed for at least six months after enrolment for all patients without a tumour to check for any subsequent malignant histology.

3.6 Data Collection

Data was collected on a structured data collection form (Appendix 3) by a single investigator. Written consent was obtained from all participants. The study received ethical clearance from the University of Witwatersrand Human Research Ethics Committee (medical). Clearance certificate number M10544 (Appendix 4).

3.7 Treatment Of Study Findings

LA cystoscopy was the reference standard, despite the limitations discussed in the literature review, due to unavailability of advanced endoscopic techniques.

A delayed-type cross-sectional principle was applied to the reference standard's results in the following way:

- Those that had had a TURBT, had their histopathology noted and correlated. If histology was benign, then these were deemed as true negatives (TN).
- Patients who were poor candidates for surgery due to concurrent health problems or advanced age and who were not biopsied yet had tumours observed endoscopically were considered positive for malignancy and designated Stage Tx and Grade Gx

In patients with a negative LA cystoscopy, the pathology database was reviewed for any subsequent bladder histology for up to six months after enrolment – any positive bladder histology in that time period was deemed a false negative (FN) by LA cystoscopy.

3.8 Outcome Measures

The primary outcome was to establish the percentage of LA cystoscopies which may be omitted if the BladderChek is positive. The secondary outcomes were the sensitivity, specificity, PPV and NPV of the BladderChek test and for UC.

3.9 Statistical Analysis

A flow diagram was constructed to summarise the flow of participants in a transparent manner. Data was recorded in Microsoft Excel. Analysis was done using MS Excel 2010, SAS version 9.1, GraphPad Software and VassarStats. Continuous data was expressed as means with standard deviations (SD). Categorical data were presented as frequencies and percentages. Where applicable, correlation between variables was determined by Fisher's exact test and plotted graphically. 2x2 contingency tables were constructed to summarise the results. The sensitivity, specificity, PPV and NPV of the BladderChek test and UC was

calculated. A p value of <0.05 was considered statistically significant. Expert statistical help was sought from Prof Geoffrey Candy.

3.10 Funding Of Study

- Participants

  All participants were recruited at time of LA cystoscopy so incurred no extra cost in participating in the study so were not compensated for their participation.

- BladderChek tests

  The BladderChek test kits were donated by Matritech/Alere for the study. The company did not have an influence on the study design, data collection nor analysis. There were no perverse incentives for the principal investigator nor conflict of interest.

- Laboratory tests and radiologic investigations

  No additional laboratory tests were performed over and above the routine tests performed for patients with haematuria or bladder tumours currently at CMJAH, therefore no additional costs were incurred by the hospital.

- Printing & stationery

  Printing and stationery costs were covered by the principal investigator and the University of Witwatersrand urology department.

CHAPTER 4

RESULTS

4.1 Study Flow Diagram

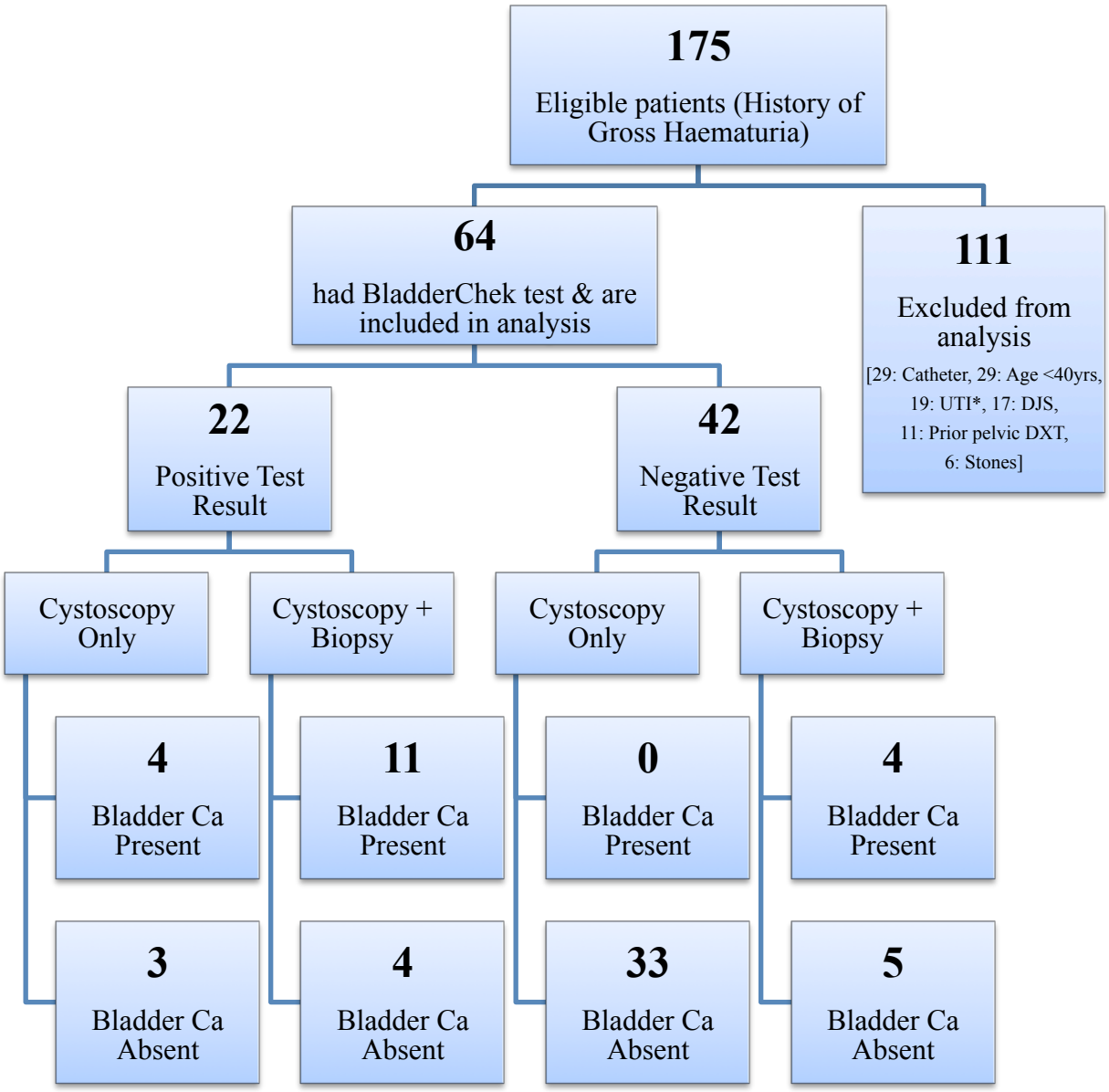
The initial plan was to recruit at least 100 patients over a 9 month period. Even though all patients who were invited to participate agreed, the pool of participants was smaller than initially projected due to the strict exclusion criteria. At interim analysis after 18 months of data collection, sufficient statistical significance was reached and the study was terminated at this point with only 74 participants due to time constraints. Unfortunately another 10 participants were excluded from final analysis due to positive urine cultures leaving just 64 participants for final analysis. 19 (29.7%) of these had BCa including 15 histologically confirmed and 4 diagnosed on cystoscopy only as detailed in figure 4.1.

4.2 The Percentage Of LA Cystoscopies That Are Unnecessary If The BladderChek Is Positive

Using the numbers from figure 4.1, within the defined group of patients who were eligible for BladderChek testing, the BladderChek correctly identified 78.9% (15/19) of all patients with BCa. As a result, approximately 12.6% (22/175) of LA cystoscopies may be avoided if patients with a positive BladderChek are assumed to have a bladder tumour and are taken directly for a planned TURBT.

Or in other words, derived from figure 4.1, it is evident that for approximately every 27 patients who presented with gross haematuria, 17 had exclusionary criteria while 10 were candidates for BladderChek testing. Amongst these 10 patients, approximately 3 had a positive BladderChek. Of these 3 patients, approximately 2 patients had a bladder tumour. Conversely, of the approximately 7 patients with a negative BladderChek, only 1 was found to have a bladder tumour on cystoscopy.

Figure 4.1 *Study flow diagram*



*UTI diagnosis: 9 based upon positive nitrates on dipsticks and 10 based upon urine microscopy and culture

4.3 BladderChek And UC Test Performance

4.3.1 BladderChek

4.3.1.1 Overall performance of the BladderChek

Table 4.1 shows that the association between NMP22 result and BCa was extremely statistically significant ($p<0.0001$). Overall, the sensitivity, specificity, PPV and NPV of the BladderChek test for BCa was 78.9%, 84.4%, 68.2%, and 90.5%, respectively (Table 4.2). The overall accuracy was 82.5%. The balanced accuracy was 81.7%.

Table 4.1 *2x2 Contingency table for NMP22 BladderChek*

	NMP22+	NMP22-
BCa (n=19)	15	4
No BCa (n=45)	7	38
Calculated two-tailed P value of contingency table using Fisher's exact test is <0.0001		

Table 4.2 *Performance of NMP22 BladderChek*

	Estimated value	95% Confidence Interval	
		Lower limit	Upper limit
Prevalence	0.296	0.192	0.425
Sensitivity	0.789	0.539	0.930
Specificity	0.844	0.699	0.930
PPV	0.682	0.451	0.853
NPV	0.905	0.765	0.969

4.3.1.2 Effect of haematuria on BladderChek performance

There were more FN's as well as FP's at increasing levels of haematuria (Table 4.3). One may calculate the BladderChek performance for two categories of dipsticks haematuria,

namely Nil/1+ and 2+/3+. The sensitivity and specificity in the former group was 66.7% and 93.9% while in the latter was 83.3% and 54.5% respectively (Table 4.4). That is the sensitivity is worse while the specificity is substantially better with less dipsticks haematuria.

Table 4.3 *Haematuria and NMP22 BladderChek results*

Urine dipsticks haematuria	True positive	True negative	False positive	False negative
NEG	1	16	0	1
1+	3	15	2	1
2+	2	2	0	0
3+	4	3	0	0
4+	4	1	5	2
Unknown	1	1	0	0
	15	**38**	**7**	**4**

Table 4.4 *NMP22 BladderChek performance stratified by degree of haematuria*

Urine dipsticks haematuria	HAEMATURIA & NMP22 SENSITIVITY/SPECIFICITY					
	Sensitivity	95% CI		Specificity	95% CI	
		Lower limit	Upper limit		Lower limit	Upper limit
NEG / 1+	0.667	0.241	0.940	0.939	0.783	0.989
2+ / 3+ / 4+	0.833	0.508	0.970	0.545	0.245	0.818

4.3.1.3 Effect of pyuria on BladderChek performance

The sensitivity and specificity of BladderChek amongst the group of patients negative for pyuria was 78.5% and 85.3% (i.e. similar to calculated performance of NMP22 overall).

4.3.1.4 Stratification of BladderChek result for stages and grades of TCC bladder

Both the BladderChek and UC were positive for the only SCC bladder in the study. The BladderChek was positive in 6 (75%), 3 (75%), and 1 (50%) of Ta, T1 and T2+ bladder tumours, respectively. The BladderChek was positive in 7 (87.5%) and 3 (50%) of LG and HG bladder tumours respectively.

4.3.2 Urine cytology

4.3.2.1 Overall performance of urine cytology

The association between the UC result and BCa was statistically significant (p=0.0065). UC results are available for only 62 of the patients since two specimens were rejected – one for leakage and one for incorrect labelling of request form. Only two (10.5%) of the patients with BCa had a positive UC. No patient without BCa had positive UC (Table 4.5).

Table 4.5 *Contingency table for urine cytology*

	Urine Cytology (n=62*)		
	Urine cytology positive	Urine cytology atypical	Urine cytology negative
BCa	2	5	12
No BCa	0	3	40
**Two specimens rejected*			
Two-tailed P value of contingency table using Fisher's exact test is 0.0065			

If one excludes the atypical UC (i.e. considers atypical as negative) then the sensitivity and specificity of UC is 10.5% and 100% respectively, however if one includes the atypical UC as positive, then the sensitivity and specificity was 36.8% and 93.0% respectively with a PPV of 70.0%. The overall accuracy for UC using the latter assumption was 75.8%. UC only detected one BCa missed by BladderChek (figure 4.2).

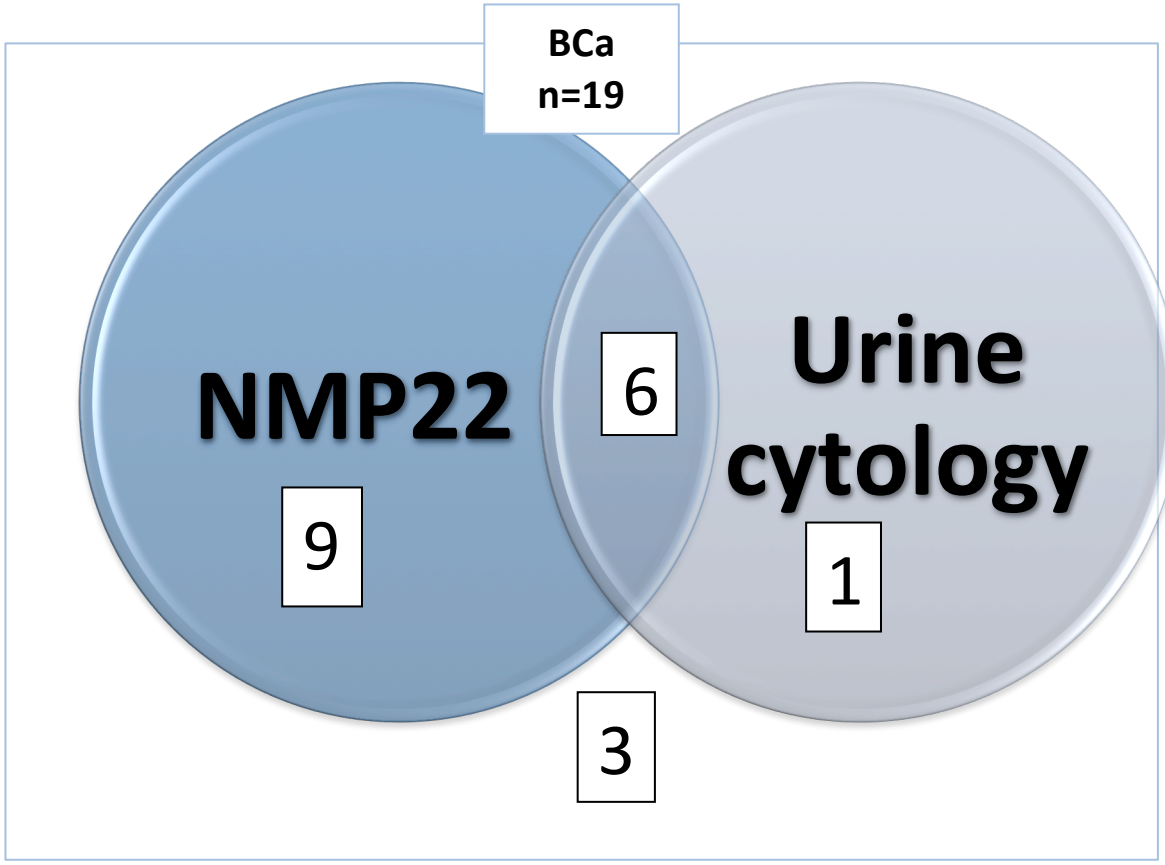


Figure 4.2 *Venn diagram of NMP22 BladderChek versus urine cytology performance*

4.3.2.2 "False positive" atypical urine cytology

Regarding the three patients without BCa on initial evaluation but atypical UC, two had TCC of urinary tract diagnosed approximately 10 months later: One was diagnosed with Ta LG TCC bladder and the other was diagnosed with upper tract TCC. The bladder tumour was in a 67 year old coloured retired carpenter. He was an ex-smoker who had stopped 30 years previously. Urine dipsticks was negative at time of cystoscopy, as was the BladderChek and cystoscopy. A subsequent TCC Ta LG was diagnosed 10 months later. He was included as a FP for UC since the study protocol stated that only tumours diagnosed within 6 months of initial cystoscopy would be considered as TP. The upper tract TCC was in an 81 year old white male with 40 pack year history who had also stopped 30 years previously. There was no CT IVP at time of cystoscopy. Urine dipsticks showed only 1+ RBC's, PSA was 0.06, BladderChek and cystoscopy were negative for bladder tumour but an upper tract TCC was diagnosed 10 months later. The third FP atypical UC was a 40 year old white male fitter and

turner. He had no smoking history, urine dipsticks were negative at time of cystoscopy, and the BladderChek test was negative. PSA was 0.54. No random biopsies were found on pathology database and no explanation was found for this atypical UC result.

4.3.2.3 Stratification of urine cytology result for stage and grade

If one includes atypical UC as positive then sensitivity was 37.5%, 75%, and 50% for Ta, T1 and T2+ bladder tumours, respectively. UC was positive in 4 (50%) and 3 (50%) of LG and HG bladder tumours respectively. Grade unknown in four patients due to cystoscopic diagnosis of tumour only and one patient had SCC BCa.

4.3.3 Comparison of BladderChek and UC performance

Table 4.6 presents a comparative summary of the BladderChek and the UC test performance.

Table 4.6 *NMP22 BladderChek compared with urine cytology performance*

	Overall sensitivity, specificity, positive and negative predictive value, and accuracy*					
	No. Pts. (prevalence)	%Sensitivity (range)	%Specificity (range)	% Pos. Predictive Value (range)	% Neg. Predictive Value (range)	% Accuracy
NMP22 BladderChek	64 (0.296)	78.9 (53.9-93.0)	84.4 (69.9-93.0)	68.2 (45.1-85.3)	90.5 (76.5-96.9)	82.8%
Pos. cytology	62 (0.306)	10.5 (1.8-34.5)	100 (89.8-100)	100 (19.8-100)	71.7 (58.4-82.2)	72.6%
Atypical + pos. cytology	62 (0.306)	36.8 (17.2-61.4)	93.0 (79.9-98.2)	70.0 (35.4-91.9)	76.9 (62.8-87.0)	75.8%
**All ranges are 95% CI*						

4.4 Comparison Of BCa Versus Non-BCa Patients Across Other Variables

4.4.1 Demographics

The mean age of the patients with BCa and those without were comparable, however clustering in the 50-70 year age range was more pronounced amongst the BCa patients (Table 4.7). The male to female ratio was even more pronounced in the BCa patients at a ratio of approximately 5:1. More than half of all participants were white. Amongst those with BCa, 68% were white while only 10% were black. Conversely, amongst those without BCa, approximately 40% were white while 40% were black. Amongst other racial groups, the proportion of participants (15-20%) was similar in both the BCa and non-BCa groups. In terms of absolute numbers, the majority of BCa patients were white males, however only 40% of white males with gross haematuria had BCa. As a proportion of patients within their respective racial and gender group, the Indian males were the highest risk with 50% of enrolled participants having BCa but the number of participants is small so this observation may be random. Non-white females and black males were the lowest risk group. (Table 4.8 and Figure 4.2)

Table 4.7 *Age, gender and race distribution*

	Cases with BCa (n=19)	Controls without BCa (n=45)
Sex, n(%)		
- **Male**	16 (84.2)	34 (75.6)
- **Female**	3 (15.8)	11 (24.4)
Mean age (SD)		
- **Overall**	62.9 (10.6)	63.5 (12.8)
- **Male**	62.7 (10.9)	63.7 (12.9)
- **Female**	68.3 (7.9)	62.8 (12.2)
Age Range		
- **Male**	42-78	40-86
- **Female**	60-79	46-78
Age group, n(%)		
- **<50yrs**	2 (10.5)	10 (22.2)
- **50-70yrs**	13 (68.4)	20 (44.4)
- **>70yrs**	4 (21.1)	15 (33.3)
Race, n(%)		
- **White**	13 (68.4)	20 (44.4)
- **Black**	2 (10.5)	18 (40.0)
- **Indian**	3 (15.8)	5 (11.1)
- **Coloured**	1 (5.3)	2 (4.4)

Table 4.8 *Race and gender distribution*

	Cases with BCa		Controls without BCa	
	Male (n=16)	Female (n=3)	Male (n=34)	Female (n=11)
White	11 (68.8)	2 (66.6)	16 (47.1)	4 (36.4)
Black	1 (6.3)	1 (33.3)	13 (38.2)	5 (45.5)
Indian	3 (18.8)	0 (0.0)	3 (8.8)	2 (18.2)
Coloured	1 (6.3)	0 (0.0)	2 (5.9)	0 (0.0)
Figure in parenthesis is a percentage of the total number in the particular gender and race group				

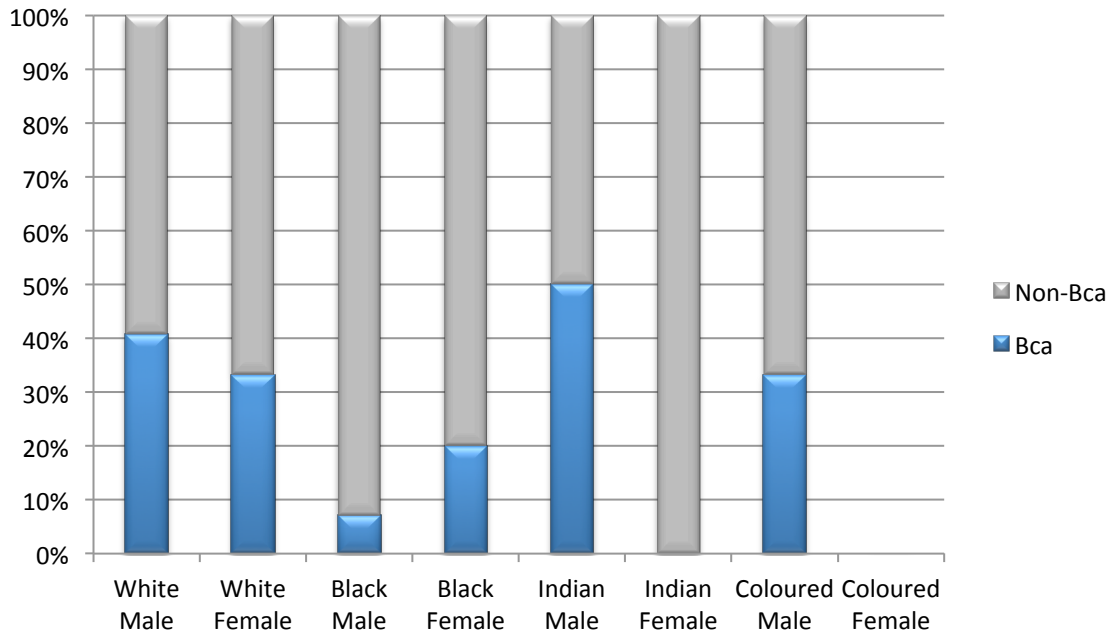


Figure 4.3 *Race and Gender distribution as a proportion*

4.4.2 Risk factors for bladder cancer

4.4.2.1 Smoking

Almost all (94.7%) of participants with BCa were current smokers or were ex-smokers. The association between smoking and BCa was extremely statistically significant (p=0.0006). The one participant with BCa who denied a smoking history, was also the eldest participant with BCa. She was a 79 year old retired black cleaner who had a negative BladderChek and UC but who was found to have a LG Ta TCC BCa. Figure 4.4 demonstrates the relationship between smoking status and BCa. The average pack years for those smokers with BCa were 38.3 years while amongst those smokers without BCa was 27.1 years. Amongst those patients with BCa who were ex-smokers, the mean and median number of years stopped was 16 and 13 respectively.

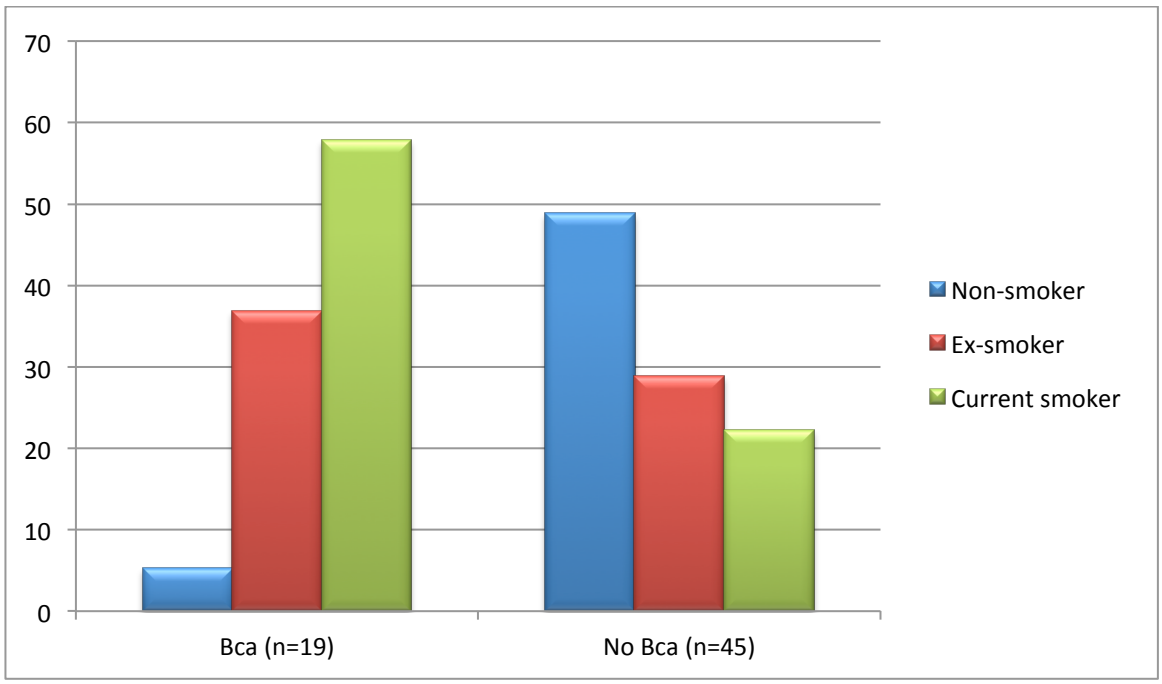


Figure 4.4 *Smoking status of participants as a percentage*

4.4.2.2 Occupation (past and current)

No occupations were identified as significant risk factors in the study (figure 4.5). The majority of participants at presentation were either retired or unemployed. There were more manual labourers in BCa group but these were simply gardeners, cleaners and construction workers rather than high risk occupations such as painters or leatherworkers. There were in fact a smaller proportion of patients with occupational diesel exhaust exposure such as garage mechanics and drivers in the BCa population than in the non-BCa occupation. In addition, factory workers were less common in the BCa than in the non-BCa group. There were two metalworkers but again both were in the non-BCa group. No textile dye or rubber factory workers were identified in the study.

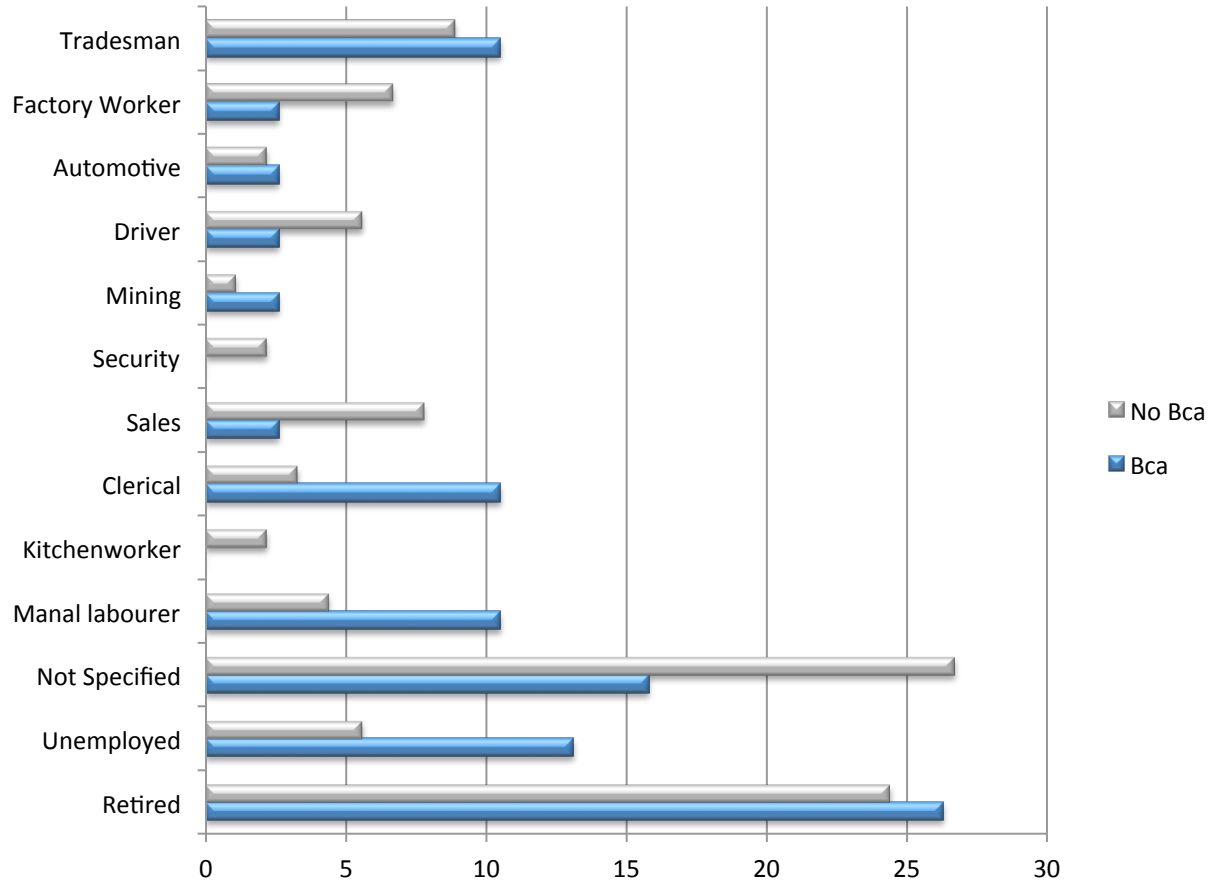


Figure 4.5 *Current and past occupations of participants*

4.4.2.3 Bilharzia

A history of Bilharzia was not found to be a significant risk factor amongst the patients with BCa (Table 4.9). The one patient with BCa and a positive history of Bilharzia is also the only patient diagnosed with SCC. As is typical of bilharzia-associated SCC, he was a black male of only 44 years of age.

Table 4.9 *History of bilharzia in participants*

	BCa (N=19) n (%)	**No BCa (N=45) n (%)**
Prior Bilharzia	1 (5.3)	4 (8.9)
No Prior Bilharzia	18 (94.7)	41 (91.1)
Two-tailed p value 1.0000		

4.4.2.4 Cyclophosphamide

No patients enrolled in study had received prior cyclophosphamide.

4.4.3 Signs and symptoms

As demonstrated in Table 4.10, there was no statistically significant association between storage symptoms, abdominal pain nor a palpable abdominal mass and BCa.

Table 4.10 *Signs and symptoms of BCa versus non-BCa participants*

	BCa (N=19) n (%)	**No BCa (N=45) n (%)**	*Two-tailed p value*
Storage Symptoms	12 (63.1)	18 (40.0)	0.1071
Abdominal pain	5 (26.3)	17 (37.8)	0.5654
Palpable abdominal mass	1 (5.3)	0 (0.0)	0.2969

The only patient in the study with an abdominally palpable bladder mass was diagnosed based upon cystoscopy only. Cold cup biopsy was not taken because he was on aspirin at the time. He was booked for admission for a TURBT but never returned to the hospital. There is no record for this patient since the LA cystoscopy. Review of his patient record reports two previous myocardial infarctions (MI) at 6 years and 5 years before this cystoscopy and no prior diagnosis of BCa. It is unclear whether his BCa was managed at another institution, whether he had another MI and was managed elsewhere, or whether he passed away before returning for the TURBT.

4.4.4 Imaging

Only 24 patients (37.5% of all study participants) had had additional imaging over above the KUB X-ray at the time of cystoscopic evaluation.

4.4.4.1 Ultrasound

Ten patients had had an USS before the cystoscopy. Ultrasonography is highly operator-dependent and unfortunately the scans were performed by various observers but this may also be more representative of the usual practice at the institution. Despite this, there was only one FN and one FP USS. The one patient with a FP USS also had a positive BladderChek and a positive cystoscopy but on histology was found to have an inverted papilloma. Accurate sensitivity and specificity of USS cannot be reported as with such a small sample size in this subgroup the 95% confidence intervals are so wide as to make the figure almost irrelevant.

Similarly, the association between the USS result and BCa was not statistically significant also probably due to small sample size (Two-tailed p value of contingency table calculated using Fisher's exact test 0.1833).

4.4.4.2 Computerised tomography

Nine patients had had a CT before the cystoscopy. Once again, accurate sensitivities and specificities are also not possible due to small subgroup of patients and again there was no statistically significant association between CT result and BCa (p=0.5238). The one patient with a FP CT also had a positive NMP22 and cystoscopy but was found to have granulomatous cystitis (non-tuberculous) on histology.

4.4.4.3 Magnetic resonance imaging

Only one patient had had an MRI before the cystoscopy. It was a FP as it was found to be cystitis on cystoscopy and histology.

4.4.4.4 Intravenous pyelography

Four patients had had an IVP before the cystoscopy. All were TN.

4.4.5 Cystoscopy

The sensitivity of WLC was found to be good in terms of no subsequent histological evidence of BCa on review of the pathology database within six months of the negative cystoscopy. The specificity of cystoscopy however was found to be problematic. Almost 18% of the patients without BCa were thought to have a possible bladder tumour or CIS on the cystoscopy but this was disproven on histological examination of a biopsy. The commonest cause of the FP on cystoscopy was found to be cystitis histologically.

4.4.5.1 BCa cases diagnosed on cystoscopy without histologic confirmation

Four patients were diagnosed on the basis of cystoscopy only and never underwent histological confirmation. All four were white males with a 30-60 pack year history of smoking. Three reported storage symptoms and two had abdominal pain. The first patient was 50 years old and had a palpable abdominal mass. He never returned to CMJAH as detailed in section 4.4.3 above. The second patient also had a large bladder tumour. His general condition was poor on admission with cachexia and bedsores. Liver metastases were confirmed on USS. During the admission, he developed oesophageal candidiasis, haemorrhagic gastritis and pneumonia. He died from a suspected aspiration after an unsuccessful resuscitation. The third patient was 70 years old with a suspicious lesion on cystoscopy. He was admitted for the TURBT but from the records seems he left the hospital soon after admission due to financial constraints and never returned. The last patient was also 70 years old, had severe emphysema and was on home oxygen. He had had a transurethral resection of the prostate (TURP) at another hospital for haematuria. Shortly after this he presented to CMJAH due to the on-going haematuria. At LA cystoscopy, an extensive sessile tumour of the anterior bladder wall was noted. During his workup as an inpatient, lung and pelvic lymph node metastases were diagnosed. He was considered too high risk for a TURBT and a poor candidate for chemotherapy. He was discharged to palliative care without histological confirmation. He died the following month after an acute deterioration and readmission.

4.4.6 Urine dipsticks

4.4.6.1 Dipsticks haematuria

A history of gross haematuria was an inclusion criterion, but due to a delay between this and the actual cystoscopy most patients had no visible haematuria at time of cystoscopy. More than half (52.6%) of the BCa patients still had 3+ or 4+ dipsticks haematuria at time of cystoscopy while most (73.4%) of those without BCa had only 1+ or no demonstrable dipsticks haematuria (figure 4.6). Considering the dipsticks result in these two categories, the association between dipsticks result and BCa was very statistically significant (Table 4.11).

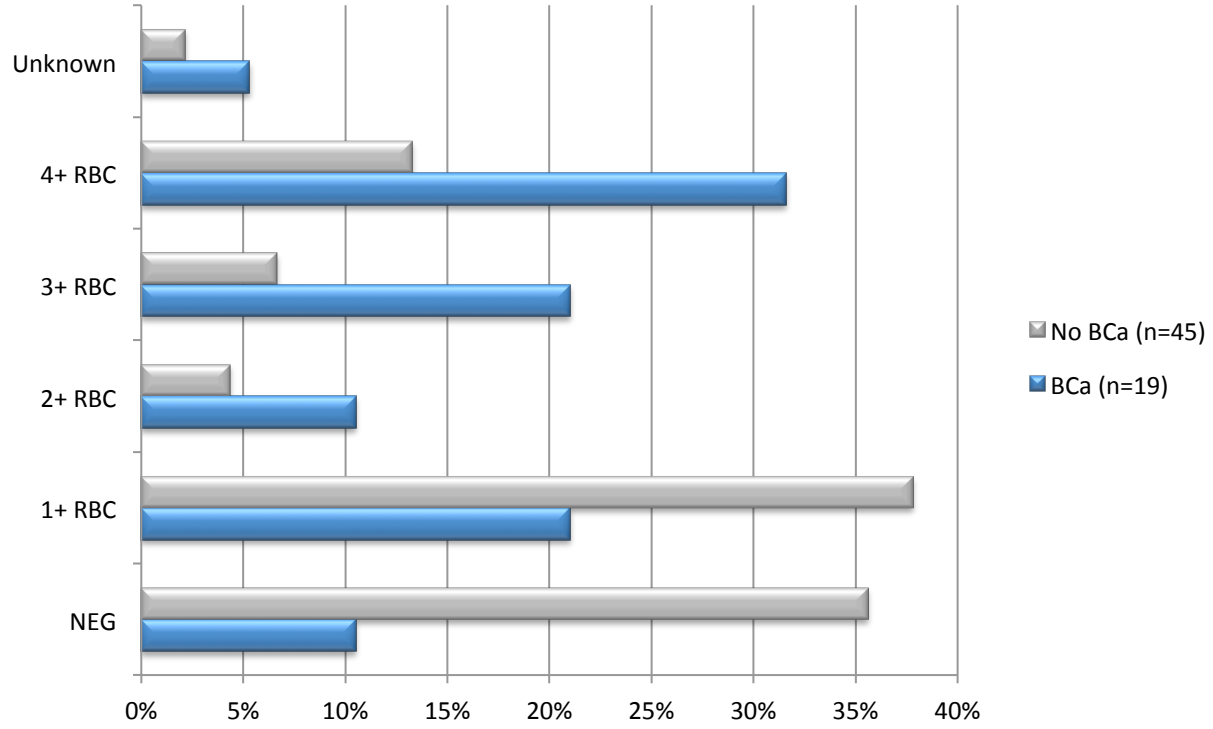


Figure 4.6 *Dipsticks haematuria results*

Table 4.11 *Dipsticks haematuria*

	Neg/1+/2+ dipstix haematuria	3+/4+ dipstix haematuria
BCa	8	11
No BCa	35	10
Two-tailed P value of contingency table 0.0087 using fishers exact test		

4.4.6.2 Dipsticks pyuria

The majority of patients (85.9%) were negative for pyuria. Because the number of patients with pyuria was so small, it is difficult to draw reliable conclusions. Nevertheless the following points are of interest:

- The proportion of BCa patients with pyuria was higher (21% versus 6.6%).

- The only patient with 3+ pyuria and BCa was also the only patient with a palpable mass.
- The only patient with 3+ pyuria and no BCa had cystitis on histology.
- All patients with low grade TCC were negative for pyuria but there was no clear association with stage on histology.
- The only patient in the study with SCC had 2+ pyuria.

4.4.7 PSA results

Eight male patients had PSA >4. Only three of these patients had prostate histology on the pathology database. Nevertheless, only two of these eight patients had a positive BladderChek and both of these patients had sufficient bladder pathology to account for the BladderChek positivity (Table 4.12).

Table 4.12 *Analysis of Patients with PSA over 4*

Participant Study number	PSA	NMP22 BladderChek Result	Bladder Histology	Prostate Histology
3	19.61	NEG	NIL	ASAP + Chronic prostatitis
13	9.60	NEG	CYSTITIS	
23	5.43	NEG	NIL	bGS 3+3
31	9.30	NEG	NIL	
33	6.81	NEG	NIL	
40	5.96	POS	CYSTITIS	bGS 3+4
63	6.58	POS	TCC T1 HG	
67	17.70	NEG	NIL	

4.4.8 Histology results

24 participants had bladder biopsies and/or TURBT:

- Fourteen were TCC Bladder: including twelve (85.7%) non-muscle invasive. Results for stage, grade, urine cytology and NMP22 BladderChek are detailed in Table 4.13.
- One was SCC Bladder: This was bilharzia-associated SCC, stage T3b, Grade G2
- Nine were negative for BCa: As can be seen from figure 4.7, the majority were cystitis. Three of the cystitis patients had a history of bilharzia exposure but no ova were noted on histology while a fourth had schistosomal cystitis on histology even though they denied bilharzia exposure and had no ova on urine microscopy.

Table 4.13 *Biopsy proven transitional cell carcinoma of the bladder*

Participant study number	Urine Cytology	NMP22	Stage	Grade
6	NEG	NEG	T1	HG
9	NEG	POS	Ta	LG
10	Atypical	NEG	T2a	HG
14	NEG	POS	Ta	LG
21	NEG	POS	T2b	HG
28	NEG	POS	Ta	LG
44	NEG	NEG	Ta	LG
47	NEG	NEG	Ta	HG
53	Atypical	POS	T1	HG
57	Atypical	POS	Ta	LG
60	NEG	POS	Ta	LG
61	Atypical	POS	T1	LG
63	NEG	POS	T1	HG
72	NEG	POS	Ta	LG

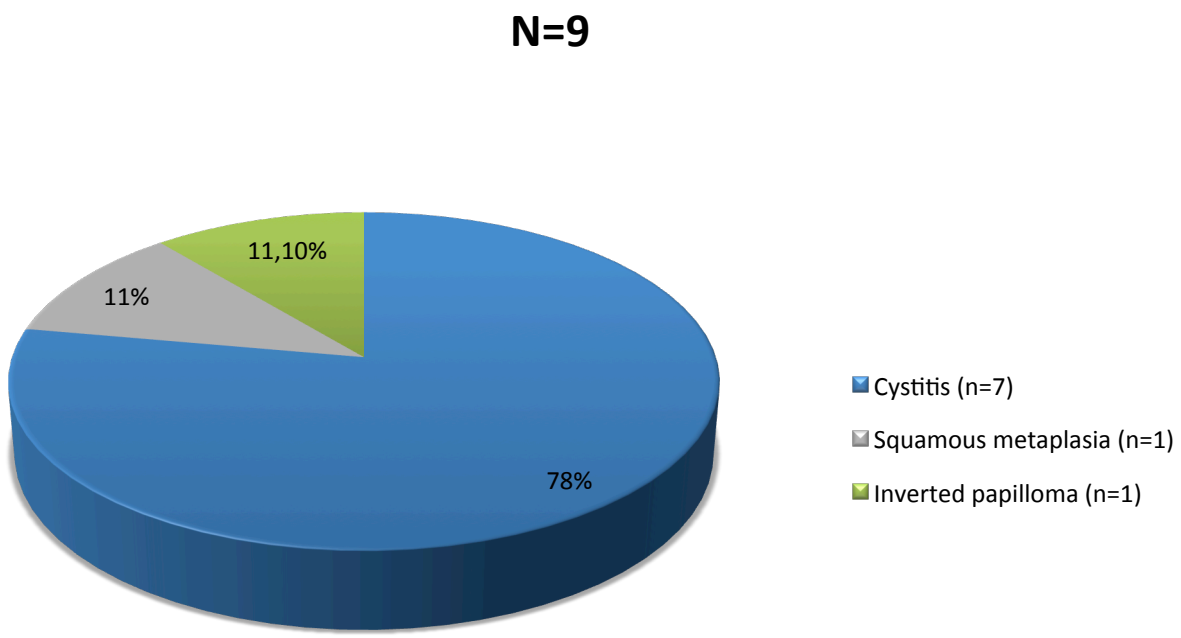


Figure 4.7 Histology of Non-malignant bladder lesions

CHAPTER 5

DISCUSSION

Major findings and importance of study

The purposes of this study were two-fold. Firstly, to establish if a positive result on the BladderChek can be used to forgo LA cystoscopy as part of the GH work-up. Secondly, to establish if UC should be abandoned in favour of the BladderChek for TCC BCa diagnosis.

This is the first study of the BladderChek to be done in South Africa. No prior published study has sought to examine whether a positive BladderChek may be used to forego LA cystoscopy during the workup for BCa. The association between the BladderChek result and BCa was extremely statistically significant. This study showed that approximately 12.6% of LA cystoscopies may be avoided if the BladderChek is applied as in this study. This would also result in 78.9% of patients harbouring bladder tumours being "fast-tracked" to GA cystoscopy and TURBT. Increasing stage and grade did not improve the BladderChek performance. Haematuria has been suggested to influence the diagnostic accuracy of the BladderChek but this study showed that the diagnostic accuracy in patients with a history of gross haematuria was comparable to many other studies. The BladderChek was slightly less specific but much more sensitive than UC for BCa resulting in a better overall accuracy. The BladderChek was found to perform sufficiently well to be considered as an alternative to UC.

Comparison to similar studies

The PPV is also known as the post-test probability of the condition after a positive test result. In this study despite all the efforts to avoid FP's, a patient in this study's highly select group only had a 68.1% chance of BCa if their BladderChek test was positive. However if the BladderChek was negative then there was a more than a 90% chance that the patient did not have BCa. Three important studies are useful for comparison of the BladderChek diagnostic performance in this study. Firstly, in the study by Sharma, et al. (1999), 12% of participants had BCa and six of the same exclusion criteria adopted in this study were used and a very

high specificity (95.6%) and PPV (87.5%) was reported. In this study, almost 30% of participants had BCa, and together with even stricter exclusion criteria, it was unexpected that the PPV and specificity were not better than this. Possible explanations for this incongruity may be differences between the quantitative NMP22 ELISA used by Sharma and the BladderChek used in this study. The cases of cystitis, especially bilharzial in our setting, may also have pushed up the number of FP BladderChek tests. Sharma also does not report the grade or stage of the tumours or the rate of CIS which has been reported to affect the performance of the NMP22 test (Shariat, et al. 2004). The second important study for comparison is the most recent meta-analysis of the NMP22 test. The calculated sensitivity of the NMP22 test was 73% based upon 834 patients while the specificity was 80% based upon 1579 patients (Lotan & Roehrborn 2003). This meta-analysis also reported better sensitivity with increasing grade and stage of TCC which is also biologically plausible but this trend was not evident in this study possibly due to the low numbers of patients when stratified into these groups. Lastly, the largest study to date was a multicentre study involving 1331 patients (Grossman, et al. 2005). A Sensitivity of 55.7% and specificity 85.7% was reported. The sensitivity was better in this study possibly because of the increased pre-test probability but it is unclear why the specificity was not better in this study despite the strict exclusion criteria.

The UC sensitivity in this study was only 36.8% (17.2-61.4%). This is less than the 52% reported in a systematic review (Glas, et al. 2003). Like other studies, urine cytology was found to be highly specific but poorly sensitive test for BCa. Unlike other studies, unexpectedly, neither UC nor the BladderChek test had better sensitivities at increasing stage or grade. UC was positive for only one tumour missed by the BladderChek, while the BladderChek detected nine tumours missed by UC.

Like most other studies white males were found to be a high risk group for TCC BCa but in addition Indian males was also found to be a high risk group. Smoking (current or past) was found to be an extremely significant risk factor for BCa. ICUD reports that 30-50% of bladder cancer is caused by cigarette smoking (eds Soloway, Carmack & Khoury 2005), yet in this study only one BCa patient in the study had no smoking history. Occupation, bilharzia exposure and cyclophosphamide were not found to be important risk factors. There was a trend towards increased storage symptoms amongst BCa, but this was not statistically

significant. Abdominal pain was not an important symptom of BCa and palpable abdominal mass was very uncommon. WLC FP rate of 18% in the study was half of that reported in a multicentre study (Sarosdy, Schellhammer & Bokinsky 2002). Patients with and without BCa were statistically significant distinct groups with respect to degree of dipsticks haematuria at time of cystoscopy (Neg/1+/2+ versus 3+/4+) with a higher proportion of BCa cases with increasing amount of dipsticks haematuria. I am not aware of this observation previously being been made in the literature. There was a trend towards increased pyuria with BCa, and a trend towards increased pyuria with increased grade but not stage. Pyuria did not seem to particularly affect the BladderChek performance but with so few patients with pyuria it cannot be completely certain that pyuria does not affect the test.

Alternative explanations for findings

The pre-test probability was manipulated to maximise the test's performance. Manipulation of a test's study population is not unusual and is frequently used in clinical practice. One of the best examples is PSA testing.

There were higher levels of dipsticks haematuria in the BCa group. Haematuria has been reported as a cause of a FP BladderChek as discussed in the literature review. This compounds the interpretation of the BladderChek accuracy since then one wonders if the haematuria itself caused the test to be positive rather than a tumour-related marker per se. Favouring this possibility is the increased number of FP's at increased levels of dipsticks haematuria noted in the study but conversely there were also more FN BladderChek results at higher levels of haematuria. An alternative explanation for this, and the differing sensitivity and specificity of the BladderChek at different levels of haematuria, is that it is simply a function of the tests performance at different pre-test probabilities.

The increased rate of bilharzia in our setting compared with Europe and the United States may have contributed to a decreased specificity due to bilharzia-associated cystitis. Five patients reported prior bilharzia exposure and four of these had positive BladderChek test. Only one had SCC bladder, while cystitis was reported on histology of another three although

schistomal ova were not noted. Another patient who denied bilharzia exposure had a positive BladderChek with a suspicious cystoscopy and schistosomal cystitis on histology. All this suggests that in our setting bilharzia is certainly an important factor that may contribute to FP's on the BladderChek test.

Study strengths

Many studies take two groups of patients – one known BCa and a second (sometimes even smaller) group of patients as controls and then examine the performance of a test. This case control study design is not appropriate for the accurate estimation of a diagnostic test's performance. A good example is the study by Jamshidian, et al. (2008) where 76 patients with known BCa and 75 patients without BCa, UTI, stones or other urinary tract malignancy were used as controls. The result was a sensitivity of 75% and specificity of 86.7% (Jamshidian, Kor & Djalali 2008). The problem with such studies is that this is not a "real life" background prevalence of the condition and certainly not one which may be obtained in practice and in which the test would ordinarily be used. This results in potential over-estimation of the accuracy of the test due to "spectrum bias" (Lijmer, et al. 1999). In this study, the BCa prevalence was not "artificial" and although patients were excluded for various reasons, the same may be easily done in clinical practice. In addition, selection bias was avoided by consecutive entry of participants and there was blinding of the BladderChek result at time of cystoscopy and when laboratory tests were performed.

Study limitations

Due to the exclusion criteria, only a third of patients with gross haematuria were eligible for study entry. This led to a relatively small sample of only 64 patients for the final analysis. These results may be considered reliable for the population that CMJAH serves but it may be less useful at other institutions even within South Africa for reasons such different populations, pretest probabilities, and rates of bilharzia. Theoretically patients may have dual pathology such as a stone or UTI as well as a bladder tumour but due to the former are excluded from having a BladderChek test. This is more a limitation of the test than this study per se. The KUB x-ray was used as a screen to exclude patients with urinary tract stones. It is well known that up to 20-30% of renal stones are radiolucent on plain x-ray. It would have been better if all patients had had a CT before the cystoscopy but unfortunately logistically in

our setting this was not possible. Voided UC, not bladder washings, were used. The sensitivity of bladder washing cytology is superior but this is not practical in daily practice so probably the voided specimen is more representative of reality. The UC was also not immediately fixed with ethanol but again this is not usually practiced at our institution either. Due to the demands of our healthcare system, visible haematuria has usually resolved by the time of the cystoscopy or even by the time of their urology outpatient appointment. The results may have been different if the test was used in patients with on-going or current gross haematuria. WLC is problematic due to sensitivity for bladder tumours generally and CIS in particular. Some may consider PDD as the current standard but this is controversial and this technology is not yet widely available including at CMJAH. Unfortunately, the number, configuration and size of tumours on cystoscopy were not recorded on the data collection sheet but may have been useful for additional analyses.

Clinical relevance and recommendations

One may imagine the use of the BladderChek being used at CMJAH in the following way. For every 27 patients presenting with gross haematuria, 17 will have exclusionary criteria while 10 will be candidates for BladderChek testing. Amongst these 10 patients, 3 will have a positive BladderChek and thus could be "spared" a LA cystoscopy and be taken directly instead for a cystoscopy under general or regional anaesthetic for a probable TURBT. Of these 3 patients, 2 patients will in fact have a bladder tumour while 1 will have an "unnecessary" anaesthetic. Conversely, of the 7 patients with a negative BladderChek, only 1 will be found to have a bladder tumour on cystoscopy. The cost of LA cystoscopies is significant for this common urological malignancy. A detailed cost analysis of this approach is beyond the scope of this study, however with such a novel approach one would need to balance the cost saving of the three LA cystoscopies that are not done, compared to the additional cost of the BladderChek tests, and the cost of the additional preoperative investigations, theatre cost and hospitalisation cost for the one patient with a positive BladderChek but who is ultimately found to not have a bladder tumour. It is likely that such an approach would be more expensive however for the patient with a bladder tumour and a positive BladderChek, it is difficult to put a monetary value on the saved morbidity of the "unnecessary" LA cystoscopy that is avoided with such an approach. In addition, a negative

BladderChek would also be reassuring for our patients and the urologists that a tumour was not being missed.

In South Africa, a UC costs more than the BladderChek test even in the public sector (refer to section 2.1.5.8 for amounts). When one considers the cost of UC and the fact that in this particular study that only one patient had a positive UC and a negative BladderChek, this does raise serious concerns as to whether we should be using the BladderChek instead. One may argue that the benefit of the UC has always been its superior specificity and that a FP BladderChek may lead to unnecessary investigations. This argument is countered by the problem of "atypical urine cytology" which is frequently reported on UC and which also requires a careful thorough investigation. For example, in this study when one examines the specificity of UC where "atypical" is also considered positive, then the specificity of UC is only slightly better than the BladderChek (93.0% versus 84.4%). The recommendation from this study would thus be to consider using the BladderChek instead of UC.

Suggestions for further research

The reasons for the close association between smoking and BCa in this study, even more so than other studies, may be worthy of further research. Possible explanations may be a higher prevalence of unfiltered tobacco smoking use or higher rate of N-acetyltransferase 2 slow acetylators in South Africa.

A detailed cost analysis of using the BladderChek as a substitute for selected LA cystoscopies would be useful. It would also be of interest to compare the accuracy of the BladderChek and the NMP22 assay in patients with a history of gross haematuria.

A study looking at General Practitioners or other primary health care providers using the BladderChek test in patients with haematuria in order to select the patients with a positive test to be "fast-tracked" for specialist assessment would be practical.

Evaluation of potential methods to discriminate the BladderChek TP's and FP's would be particularly helpful. If one analyses these two groups, more than any other demographic factor, the absence of a smoking history with a positive BladderChek, seemed to indicate a FP (Appendix 5). A study examining the use of the BladderChek nomogram to predict FP's may also be worthwhile. A nomogram has been developed for predicting probability of BCa using the BladderChek result together with other demographic factors, smoking status and degree of haematuria (Lotan, et al., 2009). It is interesting to note that the mean score on the nomogram for the TP's in this study was 200.3 with range 175-215. Ten participants or 2/3's of all TP's had a score equal to or greater than 200. Conversely, the mean score on the nomogram for the FP's was 179.3 with a range 150-195 with no FP's having a score above 195 (Appendix 6). It is enticing to consider using a nomogram such as this to predict the FP's in practice but these "cut-offs" will require further study.

CHAPTER 6

CONCLUSIONS

This is the first study of the BladderChek test in South Africa. The BladderChek test may be used to improve the workup of patients with a history of gross haematuria. Application of strict exclusion criteria may optimise the performance of this diagnostic test. This study has shown that the BladderChek is a cost-effective alternative to UC, and UC should be abandoned in favour of the BladderChek. Approximately 12.6% of LA cystoscopies may be avoided if the BladderChek test is applied as in this study. This would also result in 78.9% of patients harbouring bladder tumours being "fast-tracked" to GA cystoscopy and TURBT. In the well informed patient attending CMJAH with a history of gross haematuria who also meets the exclusion criteria used in this study, the BladderChek test may be of benefit for two reasons:

a) If it is positive then one may consider GA cystoscopy and probable TURBT as there is a 68% chance that a bladder tumour exists. This may avoid the morbidity of a "non-therapeutic cystoscopy" but this approach may increase costs overall.
b) If it is negative then one may be reassured that there is a 90% chance that there is no bladder tumour but LA cystoscopy just to make sure would still be advisable.

APPENDIX 1

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

DEPARTMENT OF SURGERY
UNIVERSITY OF THE WITWATERSRAND
DIVISION OF UROLOGY
CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC
CHRIS HANI BARAGWANETH HOSPITAL

Study Title: The predictive value of the NMP22 BladderChek test for Bladder carcinoma in patients presenting with haematuria to a South African tertiary care centre.

PARTICIPANT INFORMATION SHEET

Dear Mr/Mrs (potential study participant)

My name is Dr Mark Purdy; I am a Registrar in the Division of Urology in the Department of Surgery at Wits Medical School. I will be conducting a study on patients with haematuria (blood in the urine). Haematuria may occur due to many conditions. As your doctor has probably explained to you, one of the important causes is bladder cancer. I am conducting a study of a relatively new urine test to predict those patients with bladder tumours. The study will take place at the Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwaneth Hospital as part of a post-graduate degree (MMed).

I would like to invite you to consider participation in the study. Participation is entirely voluntary and refusal to participate will involve no penalty or loss of benefits. You may discontinue participation at any time without giving a reason. Should you agree to participate, you will be asked to sign the accompanying consent form, and you will be given a copy to take home. I may withdraw you from the study if you don't meet

certain criteria during the preliminary assessment. It is not necessary to be concerned if you are withdrawn. Nevertheless I will clearly explain whatever reason to you.

Before you agree to participate, I would like you to read through this information leaflet about the study. This leaflet will help you decide whether you would like to participate in the study. It may contain words that you do not understand. Please ask me to explain any information that you do not clearly understand. You may take home **an unsigned copy of this consent** form to think about or discuss with family and friends **before making your decision**.

The study will take place at the Charlotte Maxeke Johannesburg Academic Hospital and Chris Hani Baragwaneth Hospital. Approximately two hundred participants who meet the study criteria will be enrolled in the study. The total assessment will be completed during your usual consultation or while you are admitted in the ward and will take roughly fifteen minutes of your time. A brief medical history including your bladder symptoms and any risk factors for bladder cancer will be taken. I will examine you to see if I am able to feel any swelling in your abdomen.

If you undergo an operation to remove a bladder tumour (TURBT), the result of your rectal or vaginal examination which is routine during this procedure whilst under anaesthetic will be obtained from your operation record. I will also access and record your pathology report if you undergo a TURBT.

I will review the abdominal x-ray which is also routinely done. If you have already had an ultrasound, IVP or CT scan, I will also review this result. I will also check your PSA result which is routine for workup of men with haematuria – if this has not yet been done, I will need to take your blood in order to check it.

You will void in privacy into the container which I will give to you. I will then divide this into three specimens for four urine tests which will be performed as part of the study. Two of the tests are a urine cytology and urine m,c&s which are done routinely for all patients with bladder tumours as part of standard clinical care, regardless of whether you are enrolled in the study or not. The third urine test is a

dipsticks to confirm no suggestion of urinary infection. The last urine test is a dipsticks study called the NMP22 BladderChek test which is the subject of this study.

In summary, I am asking your consent to mainly do a new urine dipsticks test and to anonymously record the results of your other investigations.

Your participation in this study will contribute to medical knowledge that may help other patients, however, you may not benefit directly from this study. You will not be paid money to participate in the study as no extra cost will be incurred by you.

All your details will be treated with complete confidentiality, and your identity will not be made known. Data from the study will be pooled, analysed and may be presented at scientific meetings and in scientific journals. At no stage will it be possible to identify you as a participant. You will be informed of any finding of importance to your health at your next clinic visit. This clinical study protocol has been submitted to The University of Witwatersrand Human Research Ethics Committee and written approval has been granted by that Committee; approval has also been sought from the Hospital Superintendent. If you would like more information, please contact me at Chris Hani Baragwanath Hospital, Ward H4, on 011 933 8107, or Charlotte Maxeke Johannesburg Academic Hospital, Ward 386, on 011 488 3386.

Dr MR Purdy
Division of Urology
Department of Surgery
University of Witwatersrand

APPENDIX 2

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

DEPARTMENT OF SURGERY

UNIVERSITY OF THE WITWATERSRAND

DIVISION OF UROLOGY

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC

CHRIS HANI BARAGWANETH HOSPITAL

Study Title: The predictive value of the NMP22 BladderChek test for Bladder carcinoma in patients presenting with haematuria to a South African tertiary care centre.

PARTICIPANT CONSENT FORM

I hereby confirm that I have been informed by the study doctor, Dr MR Purdy about the nature, conduct, benefits and risks of the abovementioned clinical study. I have also received, read and understood the participant information sheet regarding the study. I am aware that the results of the study including personal details regarding sex, age, and diagnosis will be anonymously processed into a report. I may at any stage, without prejudice, withdraw my consent and participation in the study. I have had sufficient opportunity to ask questions and of my own free will declare myself prepared to participate in the study.

PARTICIPANT

_____	_____	_____
Print name	Signature	Date and time

I, Dr MR Purdy, herewith confirm that the above participant has been fully informed about the nature, conduct and risks of the above study.

STUDY DOCTOR

_____	_____	_____
Print name	Signature	Date and time

WITNESS/TRANSLATOR

_____	_____	_____
Print name	Signature	Date and time

APPENDIX 3

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

DEPARTMENT OF SURGERY

UNIVERSITY OF THE WITWATERSRAND

DIVISION OF UROLOGY

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC

CHRIS HANI BARAGWANETH HOSPITAL

Study Title: The predictive value of the NMP22 BladderChek test for Bladder carcinoma in patients presenting with haematuria to a South African tertiary care centre.

DATA COLLECTION SHEET

Participant Study Number:						
Age:						
Sex:	Male	Female				
Race:	Black	White	Coloured	Asian	Other:	
Symptoms:		Haematuria (gross/micro):				
		Storage Sx:				
		Abdominal Pain:				
Risk Factors:		Smoking History:		*Current Smoker:*	Yes	No
				Pack Years:		
				Tobacco (black/blonde):		
				Ex-Smoker:	Yes	No
				No. of Years Stopped:		
		Occupation:		*Present:*		
				Past:		
		Prior confirmed bilharzia:		*Yes*	*No*	Date:
		Prior cyclophosphamide:		*Yes*	*No*	Date:

Past Surgical History (including prior BCa, urinary tract manipulation or bowel interposition):				
Prior chemo- or radio-therapy (e.g. cyclophosphamide, intravesical BCG/mitomycin):				
Palpable Abdominal Mass:				
KUB:	Stones	Foreign Bodies	Other:	
Other pre-scope imaging (e.g. abdo USS, IVP, CT, MRI):				
Urine Dipsticks:				
Urine m,c&s:				
PSA				
Urine Cytology:	Positive	Negative	Atypical	
NMP22 BladderChek Test:	Time Interval		Positive	Negative
	Before Test Void:	*Before Reading Result:*		
LA Cystoscopy:				
Bladder Histology	Sub-Type:	*TCC*	*SCC*	*AdenoCA*
	T Stage:			
	Grade:	*Low*	*High*	*Other:*
Any Other Histology (e.g. prostate Bx if done; kidney if done):				

APPENDIX 4

ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Mark Purdy

CLEARANCE CERTIFICATE	**M10544**
PROJECT	The Predicative Value of the NMP22 Bladdrchek Test for Bladder Carcinoma in Patients Presenting wiht Hematuria to a South African Tertiary Care Centre
INVESTIGATORS	Dr Mark Purdy.
DEPARTMENT	Department of Surgery
DATE CONSIDERED	28/05/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 31/05/2010 **CHAIRPERSON** .....
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor : Prof M Haffajee

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX 5

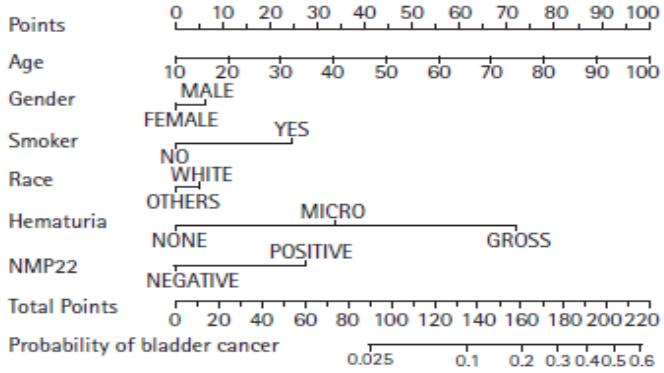
Analysis of NMP22 BladderChek true positives and false positives

NMP22 BladderChek Test		
	True Positives (n=15)	False positives (n=7)
Age, Mean(range)	62.1 (42-78)	58.0 (40-72)
Sex, M:F	14:1	5:2
Race, W:B:C	10:1:4	3:4:0
Storage Symptoms, Y:N	10:5	3:4
Abdo Pain, Y:N	4:11	2:5
Current/EX-smoker	15:0	5:2
Smoking pack years, mean	39.6	10
USS, n (pos:neg)	3 (2:1)	2 (1:1)
CT, n (pos:neg)	3 (2:1)	1 (0:1)
Urine dipsticks RBC's - **Unknown** - **NEG** - **1+** - **2+** - **3+** - **4+**	 1 1 3 2 4 4	 0 0 2 0 0 5
Urine dipsticks WBC's - **Unknown** - **NEG** - **1+** - **2+** - **3+**	 1 11 1 1 1	 0 6 1 0 0
PSA, mean (range)	1.8 (0.45-6.58)	3.3 (0.29-5.96)
Urine cytology - **Pos** - **Atypical** - **Neg** - **Rejected**	 2 4 9 0	 0 0 6 1

APPENDIX 6

NOMOGRAM and CALCULATIONS

FIG. 1. NMP22-based BC nomogram, obtained in the development subgroup of 670 patients, where age, gender, race, smoking history, haematuria and NMP22 findings define the risk of BC at cystoscopy. Nomogram instructions: To obtain the nomogram-predicted probability of BC at cystoscopy, locate patient values on each axis. Draw a vertical line to the 'Points' axis to determine how many points are attributed for each variable value. Sum the points for all variables. Locate the sum on the 'Total Points' line to assess the individual probability of bladder cancer at cystoscopy on the 'Probability of BC' line.



Mean

- TP: 200.3 (175-215) = 45% probability of BCa according to nomogram – 10 or 2/3's of TP's had total score equal to greater than 200 while none of the FP had score above 195.
- FP: 179.3 (150-195) = 35% probability of BCa – thus how many patients had total score less than 175: only one!
- TN: 156.3 (125-195) = 20% probability of BCa
- FN: 167.5 (150-190) = 25% probability of BCa – thus FN total score not actually higher than TN

NMP22 TRUE POSITIVES: +105 TO ALL AS ALL HAVE GROSS HAEMATURIA & POSITIVE NMP22

Participant study number	Age	Gender M= 10 F= 0	Smoker (>10YRS CURRENTLY OR PAST) Y= 25 N= 0	Race W= 5 O= 0	Haematuria Gross= 75 Micro= 35	NMP22 + = 30 - = 0	Total pnts	
1	50 45	M 10	Y 25	W 5	G 75	+ 30	190	Total = 3005 Mean = 200.3 Mode Median Range 175-215
8	44 40	M 10	Y 25	O 0	G 75	+ 30	180	
9	66 65	F 0	Y 25	W 5	G 75	+ 30	200	
14	72 70	M 10	Y 25	O 0	G 75	+ 30	210	
21	67 65	M 10	Y 25	W 5	G 75	+ 30	210	
28	58 55	M 10	Y 25	W 5	G 75	+ 30	200	
51	51 50	M 10	Y 25	W 5	G 75	+ 30	195	
53	42 35	M 10	Y 25	O 0	G 75	+ 30	175	
57	69 65	M 10	Y 25	O 0	G 75	+ 30	205	
60	67 65	M 10	Y 25	W 5	G 75	+ 30	210	
61	63 60	M 10	Y 25	W 5	G 75	+ 30	205	
63	78 75	M 10	N 0	W 5	G 75	+ 30	195	
65	70 70	M 10	Y 25	W 5	G 75	+ 30	215	
70	70 70	M 10	Y 25	W 5	G 75	+ 30	215	
72	64 60	M 10	Y 25	O 0	G 75	+ 30	200	

NMP22 TRUE NEGATIVES: +75 TO ALL AS ALL HAVE GROSS HAEMATURIA

Participant study number	Age 40yrs=35 50yrs=45 60yrs=55 70yrs=70 80yrs=80	Gender M= F=	Smoker Y= N=	Race W= O=	Haematuria Gross= Micro=	NMP22 + = - =	Total pnts	
2	67 65	M 10	Y 25	W 5	G 75	- 0	180	Total = 5940
3	82 80	M 10	N 0	O 0	G 75	- 0	165	
5	60 60	M 10	N 0	O 0	G 75	- 0	145	Mean = 156.3
7	50 45	M 10	N 0	O 0	G 75	- 0	130	
11	57 55	M 10	Y 25	O 0	G 75	- 0	165	Mode
13	77 75	M 10	N 0	O 0	G 75	- 0	160	
15	78 75	F 0	N 0	W 5	G 75	- 0	155	Median
17	48 45	F 0	N 0	W 5	G 75	- 0	125	Range 125-195
19	66 65	M 10	Y 25	W 5	G 75	- 0	180	
20	67 65	M 10	N 0	W 5	G 75	- 0	155	
22	40 35	M 10	N 0	W 5	G 75	- 0	125	
23	70 70	M 10	N 0	O 0	G 75	- 0	155	
25	59 55	F 0	N 0	O 0	G 75	- 0	130	
27	56 55	F 0	N 0	W 5	G 75	- 0	135	
31	62 60	M 10	N 0	O 0	G 75	- 0	145	
33	71 70	M 10	N 0	O 0	G 75	- 0	155	
34	67 65	F 0	N 0	O 0	G 75	- 0	140	
35	72 70	M 10	N 0	O 0	G 75	- 0	155	
36	70 70	M 10	N 0	W 5	G 75	- 0	160	
39	67 65	M 10	N 0	O 0	G 75	- 0	150	
41	46 40	F 0	Y 25	O 0	G 75	- 0	140	

42	69 65	F 0	N 0	O 0	G 75	- 0	140	
43	66 65	F 0	Y 25	W 5	G 75	- 0	170	
48	86 85	F 0	N 0	O 0	G 75	- 0	160	
49	72 70	M 10	Y 25	W 5	G 75	- 0	185	
50	44 40	M 10	N 0	O 0	G 75	- 0	125	
52	44 40	M 10	Y 25	O 0	G 75	- 0	150	
54	81 80	M 10	Y 25	W 5	G 75	- 0	195	
55	71 70	M 10	N 0	O 0	G 75	- 0	155	
58	81 80	M 10	N 0	W 5	G 75	- 0	170	
59	86 85	M 10	N 0	W 5	G 75	- 0	175	
62	77 75	M 10	N 0	W 5	G 75	- 0	165	
64	43 40	M 10	Y 25	O 0	G 75	- 0	150	
67	71 70	M 10	Y 25	W 5	G 75	- 0	185	
68	63 60	M 10	Y 25	W 5	G 75	- 0	175	
69	44 40	M 10	Y 25	W 5	G 75	- 0	155	
71	48 45	M 10	Y 25	O 0	G 75	- 0	155	
74	72 70	M 10	Y 25	O 0	G 75	- 0	180	

NMP22 FALSE POSITIVES: +105 TO ALL AS ALL HAVE GROSS HAEMATURIA & POSITIVE NMP22

Participant study number	Age 40yrs=35 50yrs=45 60yrs=55 70yrs=70 80yrs=80	Gender M= F=	Smoker Y= N=	Race W= O=	Haematuria Gross= Micro=	NMP22 + = - =	Total pnts	
12	40 35	M 10	Y 25	O 0	G 75	+ 30	175	Total = 1255 Mean = 179.3 Mode = 175 Median = 175 Range 150-195
24	65 60	M 10	N 0	O 0	G 75	+ 30	175	
30	68 65	F 0	Y 25	O 0	G 75	+ 30	195	
40	59 55	M 10	N 0	W 5	G 75	+ 30	175	
45	72 70	M 10	N 0	W 5	G 75	+ 30	190	
56	48 45	F 0	N 0	O 0	G 75	+ 30	150	
73	54 50	M 10	Y 25	W 5	G 75	+ 30	195	

NMP22 FALSE NEGATIVES: +75 TO ALL AS ALL HAVE GROSS HAEMATURIA

Participant study number	Age 40yrs=35 50yrs=45 60yrs=55 70yrs=70 80yrs=80	Gender M= F=	Smoker Y= N=	Race W= O=	Haematuria Gross= Micro=	NMP22 + = - =	Total pnts	SD & pVALUE
6	74 75	M 10	Y 25	W 5	G 75	- 0	190	Mean = 167.5 Mode = 165 Median = 165 Range = 150-190
10	51 50	M 10	Y 25	W 5	G 75	- 0	165	
44	60 60	F 0	Y 25	W 5	G 75	- 0	165	
47	79 75	F 0	N 0	O 0	G 75	- 0	150	

APPENDIX 7

DISCLOSURE

The BladderChek test kits were donated by Matritech/Alere for the study. The company did not have an influence on the study design, data collection nor analysis. There were no perverse incentives for the principal investigator nor conflict of interest.

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