A Multigene Urine Test for the Detection and Stratification of Bladder Cancer in Patients Presenting with Hematuria

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Purpose: We investigated whether the RNA assay uRNA® and its derivative Cx*bladder*® have greater sensitivity for the detection of bladder cancer than cytology, NMP22™ BladderChek™ and NMP22™ ELISA, and whether they are useful in risk stratification.

Materials and Methods: A total of 485 patients presenting with gross hematuria but without a history of urothelial cancer were recruited prospectively from 11 urology clinics in Australasia. Voided urine samples were obtained before cystoscopy. The sensitivity and specificity of the RNA tests were compared to cytology and the NMP22 assays using cystoscopy as the reference. The ability of Cx*bladder* to distinguish between low grade, stage Ta urothelial carcinoma and more advanced urothelial carcinoma was also determined.

Results: uRNA detected 41 of 66 urothelial carcinoma cases (62.1% sensitivity, 95% CI 49.3–73.8) compared with NMP22 ELISA (50.0%, 95% CI 37.4–62.6), BladderChek (37.9%, 95% CI 26.2–50.7) and cytology (56.1%, 95% CI 43.8–68.3). Cx*bladder*, which was developed on the study data, detected 82%, including 97% of the high grade tumors and 100% of tumors stage 1 or greater. The cutoffs for uRNA and Cx*bladder* were prespecified to give a specificity of 85%. The specificity of cytology was 94.5% (95% CI 91.9–96.5), NMP22 ELISA 88.0%, (95% CI 84.6–91.0) and BladderChek 96.4% (95% CI 94.2–98.0). Cx*bladder* distinguished between low grade Ta tumors and other detected urothelial carcinoma with a sensitivity of 91% and a specificity of 90%.

Conclusions: uRNA and Cx*bladder* showed improved sensitivity for the detection of urothelial carcinoma compared to the NMP22 assays. Stratification with Cx*bladder* provides a potential method to prioritize patients for the management of waiting lists.

Key Words: urinary bladder neoplasms; pathology, molecular

Abbreviations and Acronyms

CP = crossing point

ELISA = enzyme-linked immunosorbent assay

NMP22 = nuclear matrix protein 22

UC = urothelial carcinoma

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THE current standard of care for the detection of bladder cancer is based on flexible cystoscopy, usually with urine cytology as an adjunct. Although this combination of methods can have high accuracy, the invasiveness of cystoscopy as well as the subjectivity and modest sensitivity of cytology have stimulated the search for alternative urine tests for the initial diagnosis and monitoring of bladder cancer. Several tests have received Food

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and Drug Administration approval, although none has sufficient accuracy to routinely displace cytology.[1–6]

We recently developed a multiplex mRNA assay (uRNA) for the diagnosis and stratification of urothelial cancer that uses polymerase chain reaction amplification to quantify 4 mRNAs from unfractionated urine.[7] The method uses 2 linear discriminate algorithms to combine raw data from the individual markers. The uRNA-D algorithm is used for diagnosis and the uRNA-S algorithm predicts the likelihood that any detected cancer is high grade or has invaded through the basement membrane (stage T1 or greater).

A necessary step in the development of a diagnostic test involves evaluation in a patient cohort that closely reflects the target population for the test. Thus, we conducted a cohort study in a series of patients presenting with the most common symptom of bladder cancer, macroscopic hematuria,[8] who were to undergo cystoscopy for investigation for bladder cancer. We evaluated the original uRNA test to compare it to urine cytology and the NMP22 assays, and developed and characterized a second generation (Cx*bladder*) of the original test.

MATERIALS AND METHODS

Study Population

A consecutive series of 485 patients without a history of UC were recruited prospectively from 9 urology clinics in Australasia after ethical review and informed consent (fig. 1). Patients were eligible for the study if they had a recent history of primary gross hematuria requiring further investigation for possible urological cancer, were age 45 years or older and had no history of urinary tract malignancy. Exclusion criteria were ongoing gross hematuria less than 24 hours before investigation, prior genitourinary manipulation in the 14 days before urine collection and documented urinary tract infection.

Diagnostic Criteria

The study was restricted to patients presenting with macroscopic hematuria who underwent rigid or flexible cystoscopy. Patients were followed for 3 months for determination of UC status or alternative diagnosis. Cystoscopy was performed as part of normal clinical practice. Patients were considered positive for UC based on cystoscopic appearance and histopathological examination conducted at a local accredited laboratory. Disease stage was classified according to TNM criteria. Grade was classified according to local pathology practice using the 1973 WHO grading criteria[9] or the 1998 WHO/International Society of Urological Pathology (ISUP) consensus classification.[10] Pathology slides from resected tumor tissues were sent from the local pathology laboratories to the University of Otago for central review of tumor stage and grade. The review pathology was used in all analyses where available, otherwise local pathology was used. Further disease outcome data were obtained for participants who were positive on Cx*bladder* but negative for UC by review of available clinic records at 12 months.

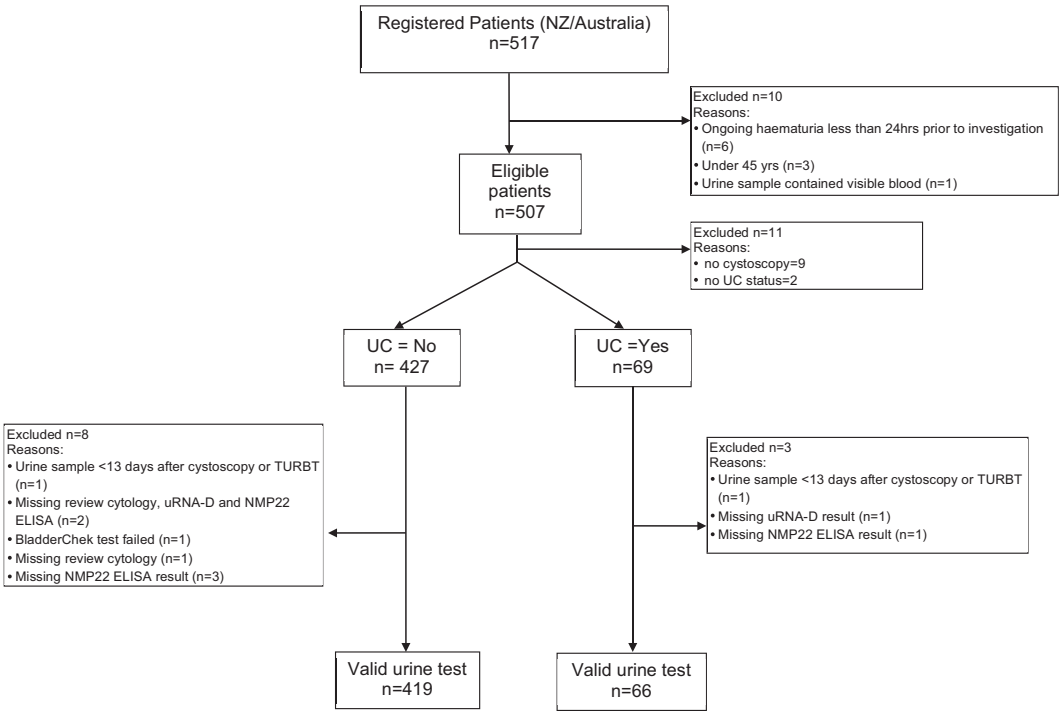

Figure 1. Flow chart showing patient recruitment/procedure and numbers. *NZ,* New Zealand. *TURBT,* transurethral resection of bladder tumor.