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Evaluation and comparison of urine markers to investigate the development of a diagnostic index for bladder cancer

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Background information and study rationale

Bladder cancer is a common problem in western countries. In Ireland bladder cancer is the 5th and 12th most common cancer in males and females respectively. On average, in Ireland between 1994 and 1996, there were 265 deaths per year from bladder cancer with age-standardized incidence and mortality rates approximately three times higher in males than females (The National Cancer Registry, 2003). In the Belfast City Hospital there are between 70-80 transurethral resection of the bladder (TURB) per year and of these 15-20 would be high risk, either TaG3 or T1G3 +/- cis (estimated current figures).

The majority of bladder cancer patients present with gross or microscopic haematuria. This is often detected by the family physician. A tumour marker that could be used to triage these patients before undergoing invasive cystoscopy, the normal out-patient investigative procedure, would be extremely cost effective and would significantly increase patient management efficiency (Glas, A. et al. 2003). In addition, a proven marker or panel of markers could be used as a screening tool for high risk asymptomatic patients. At the present time approximately 20% patients present with advanced disease and their prognosis is poorer as a result.

Over the last 10 years a large number of bladder cancer markers including BTA STAT, NMP22, telomerase and FDP, have been evaluated against the gold standard urine cytology with quite consistent results (Konety, B.R. and Getzenberg, R.H., 2001; Glas, A. et al., 2003; Bailey, M.J., 2003)(Table 1). However, many markers have low specificity and are positive in large proportions of patients with urological pathologies other than bladder cancer and in patients with urinary infections (Mukunyadzi, P., 2002; Sozen, S., 2003).

New putative markers, such as survivin (Smith, S.D. et al., 2001), burgeon the urology literature. EGF has been shown to induce expression of the matrix metalloproteinase (MMP), MMP9, in some bladder cancer cells. MMP9, itself has been proposed as a bladder cancer marker (Nutt, J.E. at al., 2003). All novel markers need to be bench marked against the high specificity of urine cytology (Pirtskalaishvili, G., Konety, B.R. and Getzenberg R.H., 1999*)* and the high sensitivity of telomerase (Glas, A. et al. 2003).

Marker	Sensitivity (%)	Specificity (%)
Urine cytology	50%; 49%; 55%	97%; 96%; 94%
BTA STAT	68%; 66%; 70%	66%; 66%; 75%
NMP22	64%; 71%; 67%	71%; 75%; 78%
Telomerase	74%; 74%; 75%	89%; 79%; 86%
FDP	68%; 68%; no data	86%; 78%; no data
Survivin	Not known	Not known
MMP 9	Not known	Not known

Table 1 Summary of results from comparative marker tumour studies

Sensitivity/Specificity data was extracted from Bailey, M.J., 2003; Konety, B.R. and Getzenberg, R.H., 2001; Glas, A.S. et al., 2003 respectively.

* Approximations based on a small pilot study.

For the purpose of this study “Sensitivity” will be defined as the percentage of individuals with the disease for whom the test is positive; the “False negative rate” as the percentage of individuals with the disease for whom the test is negative; “Specificity” as the percentage of individuals without the disease in whom the test is negative; and the “False positive rate” as the percentage of individuals without the disease in whom the test is positive (Sozen, S., 2003).

To appeal to urologists, a marker would need both 100% sensitivity (to avoid missing patients with bladder cancer) and also high specificity (to avoid patient anxiety and unnecessary invasive procedures). In assays using continuous data increasing sensitivity will always compromise specificity.

Realistically, it is unlikely that any one marker will reach the pre-requisite specificity and sensitivity required for a feasible prognostic assay. Proteomics is likely to be the way forward (Vlahou, A. 2001). In the quest to use bioinformatics to identify appropriate markers for inclusion in the predictive panel of markers, the first step is to evaluate novel transitional cell carcinoma of the bladder (TCCB) markers alongside traditional TCCB markers and to determine specificities and sensitivities for each TCCB marker in the same patient population.

Aims

1. To determine the specificities and sensitivities of two novel markers of bladder cancer (survivin and MMP9), five established TCCB markers and to establish PSA and CEA levels in 200 patients.
2. To compare specificities and sensitivities of the TCCB markers and generic cancer markers in predicting TCCB.
3. To retain a bank of urine and blood samples if the need arise to assay further markers.
4. To determine the best combination of factors for a prognostic index for TCCB.

Trial objectives and endpoints

Objectives

- To collect urine and blood samples on diagnosis and 6 months later from:
    1. 100 x in-patients with TCCB prior to transurethral resection of the bladder (TURB)
    2. 50 x in-patients with +ve haematuria/ +ve UTI / -ve cystoscopy as controls
    3. 50 x in-patients with +ve haematuria / -ve UTI/ -ve cystoscopy as controls
- To assay the following markers in blood and urine in the 200 patients
    1. c-Met
    2. Urine cytology
    3. BTA STAT
    4. NMP22
    5. telomerase
    6. MMP9
    7. survivin
    8. FDP
    9. PSA
    10. CEA
    11. S100A4
    12. FGF R3
- To benchmark the measurements of the urine markers using osmolality

Endpoints

- To determine the sensitivity and specificity of TCCB detection using each of the following markers in the 200 patients
    1. c-Met
    2. Urine cytology
    3. BTA STAT
    4. NMP22
    5. telomerase
    6. MMP9

7. survivin
8. FDP
9. PSA
10. CEA
11. S100A4
12. FGF R3

Patient selection

Participating centres

Craigavon Area Hospital, Mater Hospital, Dundonald Hospital and Belfast City Hospital

Patient identification

Patients (100) with proven bladder cancer, as defined by positive cystoscopy, about to undergo TURB, will be identified as in patients on wards at one of the 4 participating centres.

Patients (100) presenting at haematuria clinics at one of the 4 participating hospitals, without evidence of bladder cancer, i.e., patients with negative cystoscopies, will be identified as controls.

Patient population

Therefore the patient population will comprise 100 TCCB patients as group 1 and two further groups of patients of 50 each to act as controls i.e., patients without TCCB who would be expected to be seen at haematuria clinics.

200 patients presenting with haematuria as per the following groups:

1. 100 x in-patients with transitional cell carcinoma of the bladder (TCCB) prior to transurethral resection of the bladder (TURB)
2. 50 x in-patients with +ve haematuria/ +ve UTI / -ve cystoscopy as controls
3. 50 x in-patients with +ve haematuria / -ve UTI/ -ve cystoscopy as controls

Inclusion and exclusion criteria

Inclusion criteria

Patients with haematuria who have undergone cystoscopy

Patients must be able to understand the study procedures and willing to give informed consent

Exclusion criteria

Patients who have not had a flexible cytoscopy

Patients who did not present with haematuria

Patients with UTI destined to undergo TURB

Patients currently suffering from clinically evident alcoholism and or drug dependency

Patient withdrawal

If a patient wishes to withdraw from the study they will be reassured that this will have no effect on their future treatment. The samples and data records from this patient will be destroyed.

Schedule of events

Baseline Consultation

The patient will receive information prior to signing the consent form. Following consent the patient's demographics will be recorded on the Clinical information form (Table 2). Blood and urine samples will be collected by a dedicated Research Nurse and sent for analysis.

Randomisation

Not applicable

Blinding

Not applicable

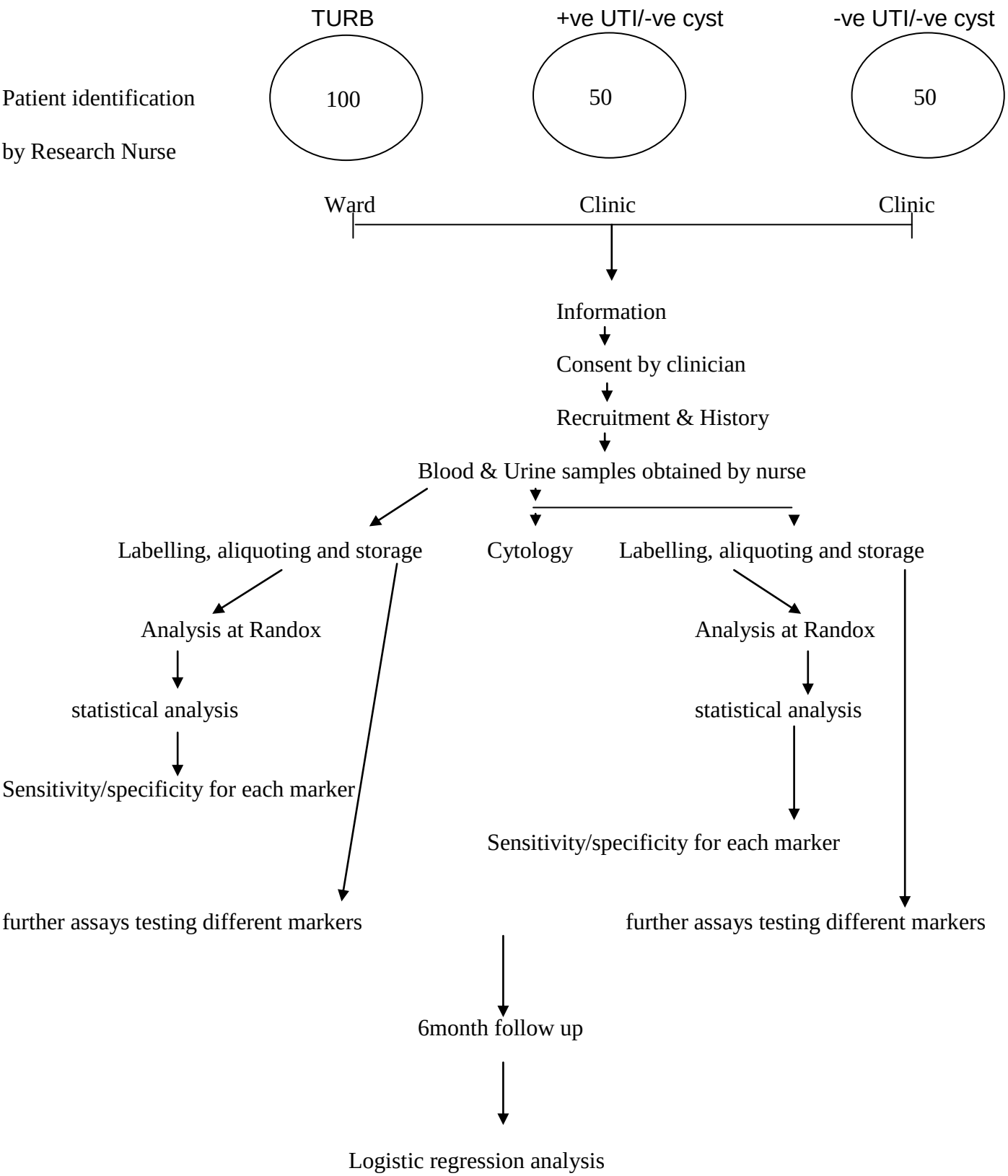

Figure 1 Orders of events
As indicated the blood and urine samples will be carefully stored so that if further appropriate markers are identified they can be assayed on the same samples.

Patient Treatment Schedule

Patients who have undergone cystoscopy will be identified at haematuria clinics or on the urology wards at one of the participating centres (Figure 1).

After the patients have been informed and consented as per ethical approval, their standard medical history will be taken and information recorded on the Clinical Information Form (Table 3) A single blood and urine sample will be obtained from each patient. In the case of the TURB group these samples will be taken on the evening prior to TURB.

Six month clinical follow-up

GROUP 1

TCCB patients will attend for 3 and 6 month flexible cystoscopy to check for recurrence as is normal practice. At six months details of recurrence and / or progression will be recorded.

Repeat blood and urine samples for marker testing will be carried out.

Any other significant changes in the patient's medical condition will be noted.

GROUPS 2 & 3

Control patients with negative cystoscopy will be invited to attend for a single visit at six months.

Follow-up urinalysis for haematuria as well as repeat blood and urine samples for marker testing will be carried out.

Blood and urine samples

Blood

Immediately after collection blood will be spun down to obtain serum. The serum will be aliquoted into nunc tubes for storage until transfer to Randox Laboratories Ltd for the analysis of bladder tumour markers (Table 3) and PSA and CEA.

Urine

Immediately after collection 5 mls of urine will be sent to the Cytopathology Laboratory in Belfast City Hospital for processing of urine cytology. An additional 5ml urine will be processed as described for serum.

ALL SAMPLES REMAINING AT THE CLOSE OF THE STUDY WILL BE DESTROYED

Marker/Test	Assay Format	Result Format
c-Met	Manual ELISA	ng/ml
BTA STAT	Lateral Flow Immunoassay	+/-
NMP22	Manual ELISA	U/ml
Telomerase	Quantitative PCR	Values from bladder cancer patients compared to normal controls
Fibrinogen Degradation Products (FDP)	Manual ELISA	ng/ml
Survivin	Manual ELISA	pg/ml
MMP 9	Manual ELISA	ng/ml
S100A4	Manual ELISA	ng/ml
FGF R3	Manual ELISA	ng/ml

Table 3 Details of Bladder Tumour Assays for Comparison

Blood and urine samples collected during the study will be stored in a dedicated –80^{o}C freezer in the Belfast City Hospital. These will be transferred on ice to the sponsoring company, Randox Laboratories, Crumlin, where they will be documented and stored until sample analysis. The above tests will be applied to the samples and results recorded.

ID code for patient	
Age	
M/F	
Occupation	
Family history of TCCB	
Previous occupational contact with chemicals, dyes.	
Smoker PAST/PRESENT	
Previous / Current Medical History <u>To include</u>: Renal Stone disease Recurrent Urine infections Any prior malignant disease History of Benign prostatic hypertrophy (BPH)	
Current Medications	
Urine Dipstick	
Urine pH	
Urine cytology	
Grade of Tumour if applicable	
Stage of tumour if applicable	
Diagnosis as at 6month follow up	
Researcher signature Date	

Table 3 Clinical information form
The nurse will record all the above information on each patient. Information will be obtained from the patient and from the patient notes

STATISTICAL ANALYSIS

Description of statistical methods to be employed

Cross-tabulations to determine the sensitivity, specificity, false positive and false negative rates for each individual marker. These are defined as follows:

Sensitivity i.e. proportion of patients prior to TURB in whom the test is positive.

False negative rate i.e. proportion of negative results that are patients prior to TURB.

Specificity i.e. proportion of individuals with UTI / -ve cystoscopy or haematuria / -ve cystoscopy in whom the test is negative

False positive rate i.e. proprotion of positive results that are individuals with UTI / -ve cystoscopy or haematuria / -ve cystoscopy / -ve UTI.

Multivariate analysis will involve logistic regression with the odds in favour of a subject being in the cystoscopy positive group being modelled as a function of (1) patient characteristics (gender, age, history of disease, familial history, smoking status etc.) and (2) the battery of markers as described above. It should be noted that sensitivity and specificity are prevalence dependent, so a method of allocating weights to each group of subjects to better reflect the patient mix to whom the battery of tests would be administered. The aim is to determine the best combination of tests and the statistic to determine this is the received operating characteristic (ROC). In view of the need for a high level of correct allocation it is unlikely that an ROC below 0.85 will be deemed clinically useful.

Number of subjects to be enrolled

200 – 100 +ve cystoscopy prior to TURB, 50 UTI / -ve cystoscopy and 50 haematuria / -ve cystoscopy / -ve UTI. This is not a comparative study so a standard power calculation is not performed. However, an approximate calculation suggests that a marker capable of a threefold

increase in the odds in favour of allocation to the +ve cystoscopy group, will have more than 80% chance of inclusion in the final model.

Level of Significance

A 5% level of significance for inclusion of any explanatory variable will be adopted. For markers a one-tailed test will be utilised (i.e. direction of marker must be intuitive).

Any criteria for termination

There is a one point in time observational study so criteria for termination is not relevant.

Suggested procedure for missing data

As ever missing data should be avoided. However, should one or two of the tests be missing for specific patients, regression methods will be applied to estimate the missing data, and predictions, suitably flagged, will be entered into the database. This is to avoid the default in the logistic regression model of 'one out - all out' which would result in complete loss of subject data.

Selection of subjects to be included in the analysis

All subjects will be included in the analysis. However to better reflect the composition of subjects to whom the battery of tests might be administered some sensitivity analysis, which will involve altering individual weights, will be undertaken.

Data handling and record keeping

Clinically pertinent patient data will be recorded as per standard urological medical history taking (see table 2). It is the investigators responsibility to ensure completion and to review and approve all patient documentation. These forms should be signed by the investigator to attest that the information

is true. At all times, the investigator has final responsibility for the accuracy and authenticity of all clinical data entered.

Record retention

To enable evaluation and / or audits from regulatory groups, the investigator agrees to keep all records, including the identity of all participants, all original signed informed consent forms, and copies of all case report forms (table 2) and details of results.

Ethics

The investigator will obtain approval of the trial protocol and any subsequent amendments from the local research ethics committee. Copies of all communications with the LREC will be retained by the investigator.

Ethical conduct of the trial

The trial will be performed in accordance with International Conference on Harmonization Good Clinical Practice guidelines and applicable regulatory requirements

Sponsor discontinuation criteria and processes

Randox reserves the right to discontinue the trial prior to inclusion of the intended number of patients, but will only exercise this right for valid scientific or administrative reasons. Randox will inform the lead investigator two months prior to any proposed discontinuation date.

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