Non-invasive diagnosis of urothelial carcinoma with urinary biomarkers and imaging after macroscopic haematuria

Background

Urothelial cancer (cancer in the bladder, ureter or renal pelvis) is amongst the most common malignancies in the world. The disease mainly affects the elderly, while it is uncommon among those younger than 50 years of age, with a median age of 73 years at diagnosis. About three quarters of patients diagnosed with urothelial cancer (UC) are men [1].

The most common symptom of UC is macroscopic haematuria. According to clinical guidelines, macroscopic haematuria should be investigated with cystoscopy and computed tomography with urographic imaging (CT urography) of the upper urinary tract (ureter and renal pelvis) [2]. In Sweden, a standardised care pathway has been in effect since 2015 which mandates this investigation for all patients older than 50 years of age with macroscopic haematuria, and which should be completed within one week. More than 90 % of UC is located in the bladder (bladder cancer), with about 2500 new cases in Sweden annually, most of which are diagnosed with cystoscopy [3].

Among patients investigated in such a standardised care pathway, 8-10 % are diagnosed with UC. Both cystoscopy and radiologic imaging of all patients with macroscopic haematuria is very resource intensive, leading to delays completing the investigations beyond the mandated week. In addition, cystoscopy is an invasive procedure with significant discomfort, and which leads to infections in up to 5 % of patients. It is therefore desirable to develop a non-invasive assay to better select patients for whom cystoscopy can be avoided, and thus be able to concentrate resources on the patients who are more likely to need timely investigations.

Non-invasive assays have been studied previously. The first was urine cytology, which shown however to have a high inter-observer variability and low sensitivity, especially for low-grade tumours. UroVysion was developed as an adjunct to cytology and is a fluorescence in-situ hybridisation (FISH) analysis of urine cytology, which detects specific genetic amplifications in urothelial cells. Sensitivity is better than for cytology, but still too low to be used to exclude further investigations, and it still suffers from a high inter-observer variability [4]. Recently, newer mRNA- and epigenetic-based assays have been reported and made commercially available. Xpert Bladder Cancer Detection is an assay based on levels of specific mRNA in urine, which are elevated in UC [5,6]. AssureMDx is an assay combining methylation and mutation of DNA in urine. Both have reported very high sensitivity, but only in retrospective series[7]. Both evaluate multiple markers and give the result as a risk of risk, so that the threshold for further action can be modified depending on the clinical setting. For screening to exclude patients for further investigation, a low threshold giving a high sensitivity but lower specificity can be utilised.

CT urography, which is the clinical standard used to detect urothelial cancer in the upper urinary tract, can also visualise urinary bladder cancer. It has previously been shown that, by timing the contrast phases appropriately, bladder cancer can be detected with high sensitivity without performing more series or using a higher radiation dose [8,9]. This protocol is therefore currently in

clinical use at most centres. However, the results have not been validated outside the initial investigation centre.

The aims of this project are therefore to evaluate the Xpert Bladder Cancer Detection test and CT urography, separately and combined, in a prospective study of patients investigated in a standardised care pathway and to create a biobank of urine samples from a well-defined cohort for further development of new biomarkers.

Hypothesis

Urine analysis using commercially available assays and new biomarkers and/or CT urography can be used to select patients with macroscopic haematuria, for whom the risk of underlying urothelial malignancy is so low that cystoscopy is not needed.

Materials and Methods

Study subjects

Consecutive patients under evaluation according to standardised care pathway for macroscopic haematuria at participating centres will be screened for participation in the study.

Inclusion criteria

- Subject ≥ 50 years of age
- Referred to participating centres for evaluation of macroscopic haematuria according to standardised care pathway
- Able to give signed informed consent

Exclusion criteria

- History of urothelial carcinoma (is also an exclusion criterion for standardised care pathway)

Candidates for enrolment will receive written information prior to visit and will give informed consent at the time of visit but before examination.

Study-specific collection

Prior to cystoscopy study subjects will give a voided urine sample and fill out a questionnaire regarding symptoms.

Subjects with suspected or clear cystoscopic or radiologic findings of urothelial cancer will have the urine sample analysed according to the protocol below. Subjects with negative findings of urothelial cancer will be randomised for analysis or not (se statistical considerations). Outcome of investigations for urothelial malignancy up to one year after enrolment will be collected retrospectively from medical records.

Urine samples collected for analysis will also be saved in a biobank for later analysis of new potential biomarkers.

Study-specific analyses

Xpert Bladder Cancer Detection (Cepheid, CA, USA; svensk återförsäljare Triolab AB, Stockholm) will be performed for all subjects with suspected or clear findings of urothelial malignancy, and for a randomised selection of subjects with normal findings. The assay is performed using a single-use cassette filled with a small sample of urine which is then inserted into a small automatic analyser, which can be placed either at an out-patient clinic or at a local laboratory. The urine sample is mixed

with a buffer and can then be stored at room temperature for up to a week before analysis, which allows for a flexible setup. The clinicians will be blinded to the results of the urine analysis.

Analysis of biobanked samples will be done in collaboration with researchers at Sahlgrenska Cancer Center, Sahlgrenska Academy, Göteborg University. Cell-free tumour DNA (cftDNA), proteins and glucosaminoglycans will be among the analysed potential markers.

Statistical considerations

The incidence of urothelial malignancy in the population undergoing investigation for macroscopic haematuria according to the standardised care pathway is 8-10 %.

The expected sensitivity of the analysed assays is 95 %, which would require 155 subjects diagnosed with urothelial malignancy to detect a non-inferiority with a null hypothesis of 90 % with alpha 0.05 and beta 0.80. A sensitivity of 90 % has been found to be the minimally acceptable standard for urine biomarkers to avoid cystoscopy [10]. The expected specificity is 60 %, which would require 148 subjects without diagnosis of urothelial malignancy to detect a non-inferiority with a null hypothesis of 50 % with same alpha and beta as above. Such sensitivity and specificity would result in a negative predictive value of 97-98 %, which is clinically acceptable, while decreasing the number of investigations needed to be performed by almost 50 %.

To allow for less than expected sensitivity, false positive investigations and drop-out, enrolment will continue until 200 cases of urothelial malignancy is detected, while 1 in 10 subjects with negative investigations will be analysed (about 180 subjects). Including equivocal findings on cystoscopy, it is expected that about 450 samples will be analysed.

Data will be analysed with calculation of sensitivity, specificity, negative predictive value and positive predictive value, as well as clinical implications, for each type of study test. The main outcome variable will be diagnosis of UC within one year of enrolment. Subgroup analysis for subjects with non-muscle invasive UC will be planned based on EORTC risk groups (low-, intermediate- and high-risk) and muscle invasive UC.

Ethical considerations and potential risks

Study assays will not affect the subjects' clinical evaluation, which will be done according to current clinical guidelines. In addition, the threshold for positive results of the study analyses will be set to maximise sensitivity, while specificity will necessarily be lower, leading to relatively low positive predictive values. Therefore, no additional investigations or follow-up will be done for subjects with positive study tests. Indeed, to minimise detection bias, both subjects and clinicians will be blinded to the results of the urine test. Study subjects will, however, be encouraged to seek medical advice in case of new episodes of macroscopic haematuria without clear cause.

The CT urography protocol, as defined in this study, is already in routine use in the participating centres. The study subjects will therefore not experience any further risks regarding radiation by participating in this study.

Time plan

Enrolment of subjects and analysis of commercial assays are expected to take 24-36 months, depending on the number of participating centres. Exploratory analyses of biobanked samples are expected to continue for several years.

Experience

The applicant is consultant urologist working mainly with diagnosis and treatment of urothelial cancer and is Principal Investigator for several ongoing clinical trials approved by the Ethics Review Board, including 1) a randomised multicentre study of robotic assisted cystectomy compared with open cystectomy, 2) an exploratory study of glucosaminoglycan profiles in urine and blood in bladder cancer and 3) an exploratory study of new biomarkers in advanced urothelial cancer.

Impact

Investigation for macroscopic haematuria is resource intensive and time consuming. If a non-invasive assay can be shown to minimise the number of full investigations needed to diagnose all or almost all cases of UC, this would improve care for all patients with UC by shortening lead-times and freeing resources for treatment instead.

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