UBC® Rapid with Reader

Urinary Bladder Cancer Detection in 10 minutes
Provides both Qualitative and Quantitative Results

High Sensitivity for CIS and Non-Invasive
High Grade Tumours

concile® Ω100
Fast, Simple & Portable.

- Rapid and instant point of care testing
- Easy to use, calibration-free
- Mobile use in clinics and hospitals
THE VALUE OF THE UBC® RAPID QUANTITATIVE MEASUREMENT OF CYTokeratins 8 AND 18 IN CLINICAL PRACTICE

Used prior to cystoscopy, it provides a useful guide to the probability of a positive finding, particularly for high grade cancer.

Used for follow-up monitoring, comparing successive readings can indicate disease progression or regression.

- Urinary bladder cancer antigen (UBC) measuring cytokeratins 8 and 18 is a useful marker for the diagnosis and clinical management of bladder cancer.¹²⁵⁸

- Sensitivity for CIS and non-Invasive high grade tumours are very high (86.9% for CIS, 30.4% for non-invasive lowgrade, 71.4% for non-invasive high grade tumours.)⁸

- Compared to urine cytology, UBC shows higher sensitivity to detect the recurrence of bladder cancer and is more reliable in guiding surveillance cystoscopy.²⁵

- The accuracy of the system is at least equivalent to a complex ELISA test.⁶

- The UBC Rapid quantitative readings showed superior performance compared with urine cytology because of improved sensitivity.⁵⁶

- TCC tumor stage and grade are directly correlated to UBC concentration. Quantified results of UBC are of prognostic relevance.⁷

- The UBC Rapid is not sensitive to the presence of blood in the urine.³

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concile® Ω 100

The reader for easy and accurate quantitative point of care testing (POCT)

Benefits at a glance
- Automatic identification of the test parameters via barcode
- Data stored in patient folders, with user name
- Calibration-free: Calibration data on SD card for each lot
- Control in every cassette
- Computer connectivity via USB cable and software for Patient Data Viewer
- Network connectivity via HL7 and GDT protocol
- Automatic Self-Check

Concile Ω100

The reader provides the concentration of cytokeratins in the sample and this is directly related to the probability of the presence of cancer

Accuracy at least equivalent to a complex USB ELISA test [6]

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Detecting of Carcinoma in situ (CIS) of Urinary bladder cancer using UBC® Rapid as tumour marker

Thorsten H Ecke (1), Christian Arndt (2), Sarah Gützlaff (3), Carsten Stephan (3, 4), Oliver Lux (1), Thomas Otto (2), Steffen Hallmann (1), Jürgen Ruttillof (1), Holger Gerullis (5)

(1) HELIOS Hospital, Department of Urology, Bad Saarow, Germany; (2) Lukaskrankenhaus Neuss, Department of Urology, Neuss, Germany; (3) Department of Urology, Charité University Hospital, Berlin, Germany; (4) Berlin Institute for Urological Research, Berlin, Germany; (5) University Hospital for Urology, Klinikum Oldenburg, School of Medicine and Health Sciences, Carl von Ossietzky University Oldenburg, Germany

Objective and Aims
Urine soluble markers should be able to ensure primary diagnosis, follow-up control and screening of high-risk populations. UBC® Rapid is a test detecting fragments of cytokeratins 8 and 18 in urine. Up to date there are no clear data for bladder cancer patients and healthy controls without disturbing factors for elevated cytokeratins in urine. Especially CIS of urinary bladder cancer is difficult to detect and has low sensitivity for cytology and even for the gold standard cystoscopy. Therefore, it is very important to find markers with a high sensitivity for CIS.

Material and Methods
Urine samples were analyzed by the UBC® Rapid point-of-care (POC) system and evaluated quantitatively using the concile Omega 100 POC reader (Fig 1, 2). Sensitivities and specificities were calculated by contingency analyses. From our study with n=452 urine samples from bladder cancer patients and healthy controls we made a stratified sub analysis for CIS of the urinary bladder (Fig 3). The cut-off value was defined at 10 µg/l.

Results
We could show that pathological concentrations of UBC rapid are detectable in urine of bladder cancer patients. The calculated diagnostic sensitivity for UBC rapid in urine was 72,4% for high grade, but only 40,7% for low grade bladder cancer. The specificity was 93,4%. Pathological levels of UBC rapid in urine are higher in patients with bladder cancer in comparison to the control group. The area under the curve (AUC) after receiver-operated curve (ROC) analysis was 0.785. Pathological values of UBC rapid in urine a higher in patients with bladder cancer in comparison to the control group.

Conclusion
UBC rapid can differ between bladder cancer patients and control group. Compared to published data of this cohort sensitivity for low grade tumours became higher. Further studies with a higher amount of samples will show how valuable these results are.

<table>
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Fig. 1: Testing UBC® Rapid
Fig. 2: concile Omega 100 reader (concile GmbH, Freiburg/Breisgau, Germany)
Fig. 3: patient characteristics and results of UBC® Rapid
Evaluation of a new quantitative point-of-care test platform for urine-based detection of bladder cancer

Rene Ritter, M.D., Jörg Hennenlotter, B.Sc., Ursula Kühs, Udo Hofmann, Stefan Aufderklamm, M.D., Pia Blutbacher, Angelika Deja, Andrea Hohneder, Valentina Gerber, Georgios Gakis, M.D., Arnulf Stenzl, M.D., Ph.D., Christian Schwentner, M.D., Ph.D., Tilman Todenhöfer, M.D.*

Department of Urology, Eberhard-Karls University of Tübingen, Tübingen, Germany

Received 15 July 2013; received in revised form 15 September 2013; accepted 25 September 2013

198 high risk patients (haematuria or irritative voiding syndromes) were included in the study; 61 clinically confirmed bladder cancer patients.

- The results of the present study show that cytokeratin concentrations determined by the Point-of-Care reader significantly correlated with those of the UBC ELISA test, implicating that the simplicity of the POC test is not associated with a loss of test accuracy.

- In our cohort, the UBC rapid determined quantitatively showed superior performance compared with urine cytology because of improved sensitivity. It is noteworthy that the combination of UBC rapid and cytology led to the detection of 12 additional tumors (including 3 High-risk tumors) compared with cytology alone.

- In case of UBC rapid determined quantitatively, not only the risk for BC in general but also the risk of having a high grade tumor (G3, Cis) increased with higher test values.

- The POC test might separate patients with Bladder Cancer from healthy patients more adequately.

- Quantitative results provide higher reproducibility and enable improved risk stratification compared with simple dichotomised (+ve or -ve) test results.
# Ordering Information

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Contact **Genesis Medical** for details of Endosheath Technology Cystoscopes and Sheaths.

Nice Guidelines recommend a biomarker as well as cystoscopy for bladder cancer detection.
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EAU 2016 presentation: Thorsten H Ecke et al (5 centres)


Preliminary Results of a Multicentre Study of the UBC Rapid Test for Detection of Urinary Bladder Cancer

Up-dated results of a multicentre-study for Urinary Bladder Cancer antigen (UBC) rapid as marker for urinary bladder cancer
Thorsten H Ecke et al, 11 World Congress on Urological Research (ESUR-SBUR15)
10-12 September 2015. Nijmegen, The Netherlands

Abstracts of Clinicals referenced in the brochure

Summary and Conclusion

Copies of complete articles referenced in this binder are available on application to Genesis Medical
Detection of Carcinoma in situ (CIS) of urinary bladder cancer using UBC® Rapid as tumour marker

Thorsten H Ecke (1), Christian Arndt (2), Sarah Gültzaff (3), Carsten Stephan (3, 4), Oliver Lux (1), Thomas Otto (2), Stefan Hellmann (1), Jürgen Rustoff (1), Horger Gerulis (5)
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Objective and Aims
Urinary soluble markers should be able to ensure primary diagnosis, follow-up control and screening of high-risk populations. UBC® Rapid is a test detecting fragments of cytokeratine 8 and 18 in urine. Up to date there are no clear data for bladder cancer patients and healthy controls without disturbing factors for elevated cytokeratine in urine. Especially CIS of urinary bladder cancer is difficult to detect and has low sensitivity for cytology and even for the gold standard cystoscopy. Therefore, it is very important to find markers with a high sensitivity for CIS.

Material and Methods
Urine samples were analyzed by the UBC® Rapid point-of-care (POC) system and evaluated quantitatively using the concile Omega 100 POC reader (Fig 1, 2). Sensitivities and specificities were calculated by contingency analyses. From our study with n=452 urine samples from bladder cancer patients and healthy controls we made a stratified sub analysis for CIS of the urinary bladder (Fig 3). The cut-off value was defined at 10 µg/l.

Results
Pathological concentrations of UBC® Rapid are detectable in urine of bladder cancer patients. The calculated diagnostic sensitivity for UBC rapid in urine was 86.9% for CIS, 30.4% for non-invasive low grade, 71.4% for non-invasive high grade, and 60% for muscle-invasive high grade tumours. The specificity was 90.9% calculated for the selected control group, and 93.8% for the whole control group (n=210). In 23 CIS the mean value of UBC® Rapid was 66.0 µg/l, for non-invasive low grade 12.2 µg/l, for non-invasive high grade 89.3 µg/l and for muscle-invasive high grade tumours 60.7 µg/l, and for healthy controls 5.9 µg/l, respectively. The area under the curve (AUC) after receiver-operated curve (ROC) analysis was 0.79 for the whole cohort.

Conclusions
Pathological values of UBC® Rapid in urine are higher in patients with bladder cancer in comparison to the control group. Sensitivity for CIS and non-invasive high grade tumours are very high. Thus, UBC® Rapid has the potential to be more sensitive and specific urinary protein biomarker for accurate detection of high-risk patients. UBC® Rapid should be added in the diagnostics for CIS and non-invasive high-grade tumours of the urinary bladder.

Table:

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Fig. 1: Testing UBC® Rapid
Fig. 2: concile® D 100 reader (concile GmbH, Freiburg/Breisgau, Germany)
Fig. 3: patient characteristics and results of UBC® Rapid
Rapid results for bladder cancer diagnosis

A new approach to point of care bladder cancer detection and risk stratification has been introduced to UK urologists, which could provide important diagnostic information to patients in a single visit.

Each year around 10,000 people in the UK are diagnosed with bladder cancer - it is the 5th most common cancer and is 3-4 times more common in men than in women. Urothelial carcinoma (alternatively urothelial bladder cancer) compromises up to 90% of all primary bladder tumours and more than 70% are non-muscle invasive bladder cancer. The recurrence rate for these patients is very high and some patients progress to muscle invasive bladder cancer or metastatic disease.

Bladder cancer is usually identified on the basis of visible blood in the urine or voiding symptoms (pain on urination) but emergency admission is a common way for bladder cancer to present, and is often associated with a poor prognosis. The National Cancer Intelligence Network (NCIN) ‘Routes to Diagnosis’ project in 2013 found that 16% of men and 24% of women diagnosed with bladder cancer in 2006-10 were diagnosed via an emergency route. Therefore, methods for early detection and regular follow-up of patients with a history of bladder cancer are of great importance in order to improve the prognosis for patients.

The most common methods for detection of bladder cancer and for the assessment of recurrence are cystoscopy and urine cytology. Cystoscopy causes pain and discomfort in patients and in cases with small flat urothelial lesions or carcinoma in situ (CIS) a diagnosis is not readily performed. Urine cytology, a non-invasive urine test, is the standard method for detection of bladder cancer and is recommended as an adjunct to cystoscopy.

However, even if cytology has the advantage of high specificity, its sensitivity for well differentiated or low-grade tumours is low. To overcome such shortcomings of the existing diagnostic methods for bladder cancer, urine tumour markers are available.

The evaluation of bladder cancer with point of care (POC) tests depends on the detection of bladder cancer associated proteins in urine samples. Common rapid tests for the early detection of bladder cancer are limited to simple positive or negative results and some of them either have low sensitivity or are influenced by the presence of blood in the urine sample (haematuria).

However, one interesting possibility is the measuring of cytokeratin fragments in urine, since elevated amounts of cytokeratin fragments are present in the urine of many individuals with bladder cancer, even at early stages of the disease.

Each year around 10,000 people in the UK are diagnosed with bladder cancer - it is the 5th most common cancer and is 3-4 times more common in men than in women.
reported to perform better than urine cytology due to improved sensitivity, in particular for low-grade tumours. Studies also show that the combination of UBC Rapid and cytology leads to detection of additional tumours as opposed to cytology alone. One clear advantage is that a test can be performed immediately and the test result made available during the patient visit. As soon as the sample is placed in the cassette, it can be inserted into a reader and 10 minutes later it will show the concentration of cytokeratins and advise whether the level indicates a normal (negative), slightly elevated (inconclusive), or elevated (positive) probability of the presence of cancer.

The UBC Rapid is also supported by a growing body of evidence. Ritter et al (2014) conducted an evaluation of the diagnostic accuracy of the POC test. A total of 198 patients with bladder cancer symptoms were included in the study. All patients received urethrocystoscopy and upper-tract imaging. Urine samples were analysed by the UBC Rapid POC system and evaluated both visually and quantitatively using a POC reader.

For visual evaluation, different thresholds of band intensity were considered for determining a test positive were applied. Moreover, the UBC enzyme-linked immunosorbent assay (ELISA), urine cytology, and the nuclear matrix protein (NMP) 22 BladderChek were performed. Sensitivities and specificities were calculated by contingency analyses. Optimal cut-offs of quantitative tests were determined by receiver operating characteristic curves.

The authors reported that a total of 61 patients (30.8%) were diagnosed with bladder cancer. Visual evaluation of the UBC revealed sensitivities of 38.1% to 71.4% with corresponding specificities of 54.1% to 89.1%, dependent on the threshold of band intensity applied. The quantitative UBC Rapid showed a sensitivity of 60.7% and a specificity of 70.1% at optimal cut-off.

A constant increase of both the probability of bladder cancer and high-risk bladder cancer with increasing UBC Rapid values was observed. UBC concentrations determined by the reader significantly correlated with the UBC ELISA (P<0.001). The UBC ELISA, NMP22 BladderChek and cytology showed sensitivities of 48.3%, 16.4%, and 51.7% with specificities of 71.3%, 95.3%, and 78.1%, respectively.

The authors concluded that the UBC Rapid, in combination with a quantitative POC-reader system, for the first time enables quantitative determination of a bladder cancer marker under POC conditions. Diagnostic accuracy was found to be at least equivalent to elaborate ELISA-based measurement. The authors added that quantitative use of the UBC Rapid test facilitates risk prediction compared with conventional non-quantitative dichotomised POC testing.

Ecke et al (2015) also recently presented the first results of a multi-centre study using UBC Rapid. Clinical urine samples from 92 patients with urinary bladder tumours (45 low-grade and 47 high-grade tumours) and from 33 healthy controls were used. Urine samples were analysed by the UBC Rapid system and evaluated both visually and quantitatively using an Omega 100 POC reader.

The researchers found that pathological concentrations of UBC Rapid are detectable in urine of patients with bladder cancer and reported that the diagnostic sensitivity was 68.1% for high-grade, but only 46.2% for low-grade tumours. The specificity was 90.9%. The area under the curve (AUC) after receiver-operated curve (ROC) analysis was 0.733. They concluded that UBC Rapid can differentiate between patients with bladder cancer and controls, adding that further studies with a greater number of patients will show how valuable these results are.

In 2014, Ludecke et al further presented the results of an evaluation of the UBC Rapid in diagnosing bladder cancer, as well as relevant clinical interferences, in a poster presentation at the Societe Internationale d’Urologie (SIU) conference held in Glasgow. The researchers described the system as a ‘powerful’ bladder cancer diagnostic test, suitable for out-patient support, and reported that an interference of blood in urine with UBC Rapid can be excluded. The additional benefits of easy handling and rapid results were also highlighted by the authors.

This followed an earlier study by Ludecke et al (2012) which evaluated the suitability of three POC test systems - UBC Rapid, NMP22 BladderChek and BTA stat - with respect to interference due to blood contamination in urine samples. Urine samples were obtained from voluntary asymptomatic individuals without a history of bladder cancer. A specimen negative in all test systems was selected for further study. This sample was treated with fresh heparinised blood in a 1:10 ratio and then titrated in a dilution series. All the urine samples and their consecutive test results were photographed and a urinalysis was performed on each sample. The UBC Rapid and NMP22 BladderChek did not show a false-positive result due to blood contamination in any of the samples. In contrast, with the BTA stat testing system, false-positive results were obtained from all samples with macrohaematuria and with densities up to 150 erythrocytes/µl - indicating a suspected tumour, when the sample was actually proven to be tumour free.

The authors concluded that, for the primary diagnosis of bladder carcinoma, neither the UBC Rapid nor the NMP22 BladderChek POC test systems are sensitive to the presence of blood in the urine.
Urine, whereas BTA stat consistently yields false-positive results due to cross-reactivity to macrohaematuria and microhaematuria up to a density of 150 erythrocytes/µL.

Sanchez-Carbayo et al (2001) also evaluated the use of serial urinary tumour markers to individualise intervals between cystoscopies in the monitoring of patients with bladder carcinoma. The study comprised 1185 urine samples belonging to 232 patients with a previous bladder carcinoma: 106 patients under follow-up (Group 1) and 126 bladder carcinoma patients receiving intravesic instillations (Group 2).

Patients were monitored with urinary tumour markers during a one-year follow-up period. Urine samples were collected before cystoscopies and in the intercystoscopic periods for patients in Group 1 and before intravesic instillations for patients in Group 2. Urinary bladder carcinoma antigen (UBC), CYFRA 21-1 and nuclear matrix proteins (NMP22) were measured by immunoassays.

Monitoring of the disease with urinary tumour markers could detect recurrence sooner than scheduled cystoscopies in 27 patients (87%) for UBC, 27 patients (87%) for CYFRA 21-1 and 26 patients (84%) for NMP22 out of 31 Group 1 patients who recurred; and in 16 patients (67%) for UBC, 17 patients (71%) for cytokeratin fragments (CYFRA 21-1), and 13 patients (54%) for NMP22 out of 24 Group 2 patients who recurred.

The most relevant finding was that persistence of negative urinary markers during follow-up was largely indicative of disease free status in 65 of 75 (87%) patients of Group 1 and 31 of 102 (30%) cases of Group 2. Although false positive results were present, they were mainly associated with sporadic urinary tract infections in 10 of 75 (13%) cases of Group 1 and 36 of 102 (35%) patients of Group 2; and with urine samples collected in the first two months at the beginning of intravesic therapy in 35 of 102 patients (34%) in Group 2.

The authors concluded that monitoring of bladder carcinoma patients with serial urinary tumour markers could anticipate detection of recurrence. Persistent negative results might postpone and reduce the number of cystoscopies. Once the limitations leading to false positive results are controlled by urinalysis, and by starting sample collection when basal levels are reached in patients with intravesic therapy, urinary tumour markers may eventually individualise the intervals between cystoscopies in the surveillance of patients with bladder carcinoma.

**UK launch**

This growing body of research indicates that UBC Rapid could provide a valuable tool for the cost-effective detection of bladder cancer in a POC setting. Genesis Medical recently introduced this new approach to UK urologists, for the first time; at the British Association of Urological Surgeons (BAUS) annual conference held in Manchester, June 2015.

Robin Penberthy, managing director, Genesis Medical, commented: “NICE guidelines recommend a diagnostic tool such as a biomarker in addition to cystoscopy. What is exciting is that the UBC Rapid is the fastest way to have a result and is more sensitive than cytology and other methods, which usually require the patient to return for subsequent visits because results are available days or even weeks after the sample is sent to the lab.

‘With this new approach, a complete diagnosis can normally be made, with confidence, in just one visit. If cancer has been detected, the quantitative level of cytokeratin concentration given by the reader, during successive follow-up visits, can be a useful indication of the progress of the disease.

**References**


Preliminary Results of a Multicentre Study of the UBC Rapid Test for Detection of Urinary Bladder Cancer

THORSTEN H. ECKE1, CHRISTIAN ARNDT2, CARSTEN STEPHAN3,4, STEFFEN HALLMANN1, OLIVER LUX1, THOMAS OTTO3, JÜRGEN RUTTLLOFF1 and HOLGER GERULLIS5,1

Author Affiliations

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4. 4Berlin Institute for Urological Research, Berlin, Germany
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Abstract

Background/Aim: UBC Rapid is a test detecting fragments of cytokeratins 8 and 18 in urine. These are cytokeratins frequently overexpressed in tumor cells. We present the first results of a multi-centre study using UBC Rapid in patients with bladder cancer and healthy controls. Materials and Methods: Clinical urine samples from 92 patients with tumors of the urinary bladder (45 low-grade and 47 high-grade tumors) and from 33 healthy controls were used. Urine samples were analyzed by the UBC Rapid point-of-care (POC) system and evaluated both visually and quantitatively using a concile Omega 100 POC reader. For visual evaluation, different thresholds of band intensity for considering a test as positive were applied. Sensitivities and specificities were calculated by contingency analyses. Results: We found that pathological concentrations by UBC Rapid are detectable in urine of patients with bladder cancer. The calculated diagnostic sensitivity of UBC Rapid in urine was 68.1% for high-grade, but only 46.2% for low-grade tumors. The specificity was 90.9%. The area under the curve (AUC) after receiver-operated curve (ROC) analysis was 0.733. Pathological levels of UBC Rapid in urine are higher in patients with bladder cancer in comparison to the control group (p<0.0001). Conclusion: UBC rapid can differentiate between patients with bladder cancer and controls. Further studies with a greater number of patients will show how valuable these results are.

It is known that urinary bladder cancer has a high rate of recurrence; a significant number of non-invasive tumors will progress to muscle-invasive disease. pTa tumors, the most common form of non-invasive bladder cancer, are mostly low-grade and often recur, but rarely progress to invading the lamina propria (pT1) and becoming muscle-invasive tumors (pT2–T4), whereas carcinoma in situ (Cis) are always high-grade and are thought to be the most common precursor of invasive tumors. Tumour grade and stage are not accurate in predicting the biological behaviour and thus guiding the choice of treatment, especially in high-risk cases (1–4).
Bladder cancer is one of the most expensive malignancies in Western countries; the cost from diagnosis to death was calculated as the fifth highest of all tumor types (5,6). Therefore bladder cancer markers are needed to reduce cost intensity and the need for painful examinations such as cystoscopies. A definition of risk groups could help to determine which treatment is the best for the patient.

It seems that a urinary-based assay might detect the presence of bladder cancer, because the disease is in contact with urine constantly, malignant cells are shed into the urine, and it is likely that urine contains the carcinogens producing the malignancy. But it is unlikely that one single molecular marker can detect all bladder cancer accurately.

Monitoring of patients with non-invasive bladder cancer is necessary due to its recurrence rate and progression risk. It seems attractive to use urine based tests to detect or exclude tumor recurrence. Diagnosis and aftercare are still based on urine cytology and diagnostic cystoscopy. New markers in this field might allow for actual after-care strategies to be modified, even simplified. Cost, patient load and cost of cystoscopies in aftercare are important reasons for the use of urinary tests. Nowadays there are other urine-based possibilities for bladder cancer detection. Some of these methods have a higher specificity and sensitivity than classical urine cytology and can be important for screening (7).

Cytokeratins are intermediate filaments of the cytoskeleton. The main function of cytokeratins is to enable cells to withstand mechanical stress. Twenty different cytokeratins have been identified in humans, and cytokeratins 8, 18, 19, 20 have been identified as being important in bladder cancer (8).

Cytokeratin 20 is expressed in transitional cell carcinoma by all cells, in normal urothelial cells only by the cover cells. It can be measured in higher levels of tumors. The other cytokeratins such as 8, 18, and 19 are expressed at higher levels on urothelial cells and may be elevated due to a higher cell turnover rate. Reverse transcriptase-PCR (RT–PCR) or immunocytochemistry was used to measure cytokeratin 20 in exfoliated cells. The sensitivity of cytokeratin 20 in all used methods ranges between 78% and 87%. The specificity ranges between 55% and 80% (9–11).

Fragments of cytokeratin 8 and 18 can be measured qualitatively with the UBC Rapid test. The evidence is low for low-grade tumors and benign urological diseases (12,13). The urine soluble cytokeratins 8 and 18 can also be detected quantitatively with monoclonal antibodies using sandwich–ELISA.

The aim of the present study was to evaluate the diagnostic sensitivity and specificity of UBC Rapid in patients with urinary bladder cancer comparing with healthy individuals.

**Materials and Methods**

**Patients.** For this prospective study, 92 patients with confirmed bladder cancer and 33 healthy controls were included between January and September 2014 at the Department of Urology, HELIOS Hospital Bad Saarow (study centre I) and Lukaskrankenhaus Neuss (study centre II), Germany. The study was approved by the local Institutional Review Board of Landesärztekammer Brandenburg. All patients with confirmed bladder cancer underwent cystoscopy, bladder ultrasound, and transurethral resection of bladder tumor in case of abnormal findings. Exclusion criteria were any kind of mechanical manipulation (cystoscopy, transrectal ultrasound, and catheterization) within 10 days before urine sampling. Other exclusion criteria were benign prostate enlargement, stones in the urinary tract, other tumor diseases, diabetes mellitus, infections, and pregnancy.
Procedure. Midstream urine was collected in a sterile plastic container and processed subsequently. Urine samples were analysed by the UBC Rapid Test (concile GmbH, Freiburg/Breisgau, Germany). All tests were carried out as advised by the manufacturer’s instructions. Firstly the results of the UBC Rapid Test were evaluated visually. The presence of a test band after 10 minutes of incubation was subdivided into three categories (no band, weak band intensity, and strong band intensity). After visual evaluation, the test cartridges were analyzed by the photometric point-of-care (POC) system concile Omega 100 reader (concile GmbH, Freiburg/Breisgau, Germany) for quantitative analysis. The Omega 100 reader illuminates the test field with a complementary coloured light to reduce interference in the analysis. The built-in charge-coupled device-matrix sensor takes a photograph of the light reflected, which is analysed by the device.

Statistical analysis. Statistical calculations were carried out with MedCalc version 12.2.1 (MedCalc Software) for ROC curve analysis. The area under the curve (AUC) ROC curves were estimated according to the method of Parker and DeLong (14). ROC curves were used to compare specificities at given sensitivities. $p$-Values of less than 0.05 (2-sided test) were considered significant.

Table I. Number of cases at the HELIOS Hospital Bad Saarow (study Centre I) and Lukaskrankenhaus Neuss (study Centre II).

<table>
<thead>
<tr>
<th></th>
<th>Study centre I</th>
<th>Study centre II</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>47</td>
<td>45</td>
<td>92</td>
</tr>
<tr>
<td>Low-grade</td>
<td>26</td>
<td>19</td>
<td>45</td>
</tr>
<tr>
<td>High-grade</td>
<td>21</td>
<td>26</td>
<td>47</td>
</tr>
<tr>
<td>Control group</td>
<td>21</td>
<td>11</td>
<td>33</td>
</tr>
</tbody>
</table>
Results

A total of 125 patients were included in the study, 92 with confirmed bladder cancer and 33 healthy controls with no history of bladder cancer. The median age of the study population was 73 (range=25–92) years. Out of these patients, 97 (77.6%) were men and 28 (22.4%) were women. Among the 92 patients with confirmed bladder cancer, 45 had low-grade and 47 had high-grade BCa; 71 (77.2%) had non–muscle-invasive bladder cancer (pTa and pT1 tumors), 21 (22.8%) had stage pT2–4. Carcinoma in situ (Cis) was detected in 10 cases (10.9%). A total of 18 (19.6%) patients had G1 tumors, 46 (50%) G2, and 28 (30.4%) had G3 tumors.

The number of patients and healthy controls are listed in Table 1 for study Centre I (HELIOS Hospital Bad Saarow) and study centre II (Lukaskrankenhaus Neuss). Both groups enrolled a similar number of patients in the study.

Test performance. Visual inspection of the cartridge revealed intermediate and strong test band intensity in 11 and 42 patients, respectively. In 71 samples, no band was visible.

Sensitivity was calculated as 53.3%, specificity was 90.9%. The AUC of the quantitative UBC Rapid Test using the optimal threshold obtained by ROC analysis (cut-off=9.1 µg/l) was 0.733.

ROC curve analysis is shown in Figure 1. More details about the calculation of AUC are given in Table II. After these procedures the cut-off value for UBC Rapid was set to 9.1 µg/l for this study. The statistical significance of differences in detection between patients and healthy controls was $p<0.0001$.

Discussion

Current guidelines recommend the use of urine markers only as an adjunct to cystoscopy owing to their limited accuracy (15–17). Newer tests, such as FISH and immunocytology, have shown improved sensitivity compared to cytology (2, 18,19), but they are complex to perform and require specialized laboratory facilities. POC tests for bladder cancer have been introduced, aiming to overcome complex testing and high costs and do provide a cost– and time–effective adjunct to cytology. The main limitations of most of these tests are their relatively high rate of false–positive tests (due to infection, mechanical manipulation, other tumor diseases, diabetes mellitus, and the presence of stones) and the semi–quantitative evaluation process. In general, bands on lateral flow test cassettes are evaluated visually and compared with a control band. As it is not possible to determine an exact threshold for test positivity, this process leads to considerable intra observer and interobserver variability, which might contribute to the broad range of test results in prior studies (20,21).

Table II. Area under the curve (AUC)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>AUC</td>
<td>0.733</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.045</td>
</tr>
<tr>
<td>95% Confidence interval</td>
<td>0.646-0.808</td>
</tr>
<tr>
<td>z Statistic</td>
<td>5.175</td>
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<tr>
<td>Significance level (area=0.5), p-value</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$^a$(14); $^b$Binomial exact.
The aim of the present study was to evaluate the performance of a POC test for bladder cancer, which can be determined quantitatively by the use of a special POC test reader. The results of the present study show that cytokeratin concentrations determined by the POC reader significantly correlated between patients with bladder cancer and healthy controls. The AUC as a parameter of diagnostic quality of the quantitative UBC Rapid Test was calculated with 0.733 based on a cut-off value of 9.1 µg/l. The test accuracy of a manual (visual) analysis of the UBC Rapid Test strongly depended on the intensity of test bands required for a positive test. When considering test bands with strong and intermediate intensity as positive tests, the manual analysis of the UBC Rapid gave similar results to those with the quantitative determination. This might raise concerns whether quantitative analysis of UBC Rapid is really necessary to achieve good test accuracy. We also showed that the result of the UBC Rapid Test is a continuous parameter and the higher the value, the more likely is the existence of bladder cancer (13). The dichotomization of a continuous parameter leads to a significant loss of information. The semiquantitative categorization of test band intensity of POC test cassettes with different thresholds for test positivity is rarely performed. Therefore, POC tests are mostly performed as tests with dichotomized results.

Neither for UBC Rapid Test nor for other POC tests for bladder cancer (such as BTA and NMP22) do manufacturers provide protocols or images enabling adequate semiquantitative assessment. In the case of UBC Rapid Test determined quantitatively, not only did the risk for bladder cancer in general increase, but also the risk of having a high-grade tumor (G3, Cis) increased with higher test values. This feature underlines the significance of a quantitative consideration of the UBC Rapid Test, as is also the case for other quantitative urinary markers (22). A dichotomized use of this marker is not able to fully-exploit its predictive potential. When using the POC reader, interpretation of the UBC Rapid results needs to include the absolute value of the test and not only a stratification into a positive or negative result. Otherwise, there might be no additional benefit of performing the POC test quantitatively.

A study conducted by Hakenberg et al. showed a sensitivity and specificity of 64.4% and 63.6%, respectively, for UBC Rapid in a collective of 181 patients of which 90 had bladder cancer (23). Mian et al. found a sensitivity for UBC rapid of 66.0% with a specificity of 90.0% (24). However, their collective consisted of 68% patients in follow-up after transurethral resection, which might...
account for the difference compared with our study. Schröder et al. reported a sensitivity and specificity for UBC rapid of 35.6% and 75.0% (12).

The optimal use of the UBC Rapid Test in daily practice or (if implemented) in one-stop haematuria clinics remains to be defined. The test might be of particular interest for Institutions not having access to elaborate tests, such as FISH or immunocytoology. In contrast to dichotomized urinary tests, its quantitative character enables risk stratification for bladder cancer to be performed based on the absolute UBC Rapid value. A positive UBC Rapid result should not inevitably lead to cystoscopy. The test results should rather be combined with clinical information (such as haematuria, age, smoking status, and possible exogenous factors, such as infection etc.) and the result of urine cytology for optimal interpretation and clinical decision-making. Thereby, the test might not only contribute to improved detection of bladder cancer, but also to improved prediction of high-risk tumors, which has also been shown for other quantitative protein-based urinary tests (25). One approach to objectify risk stratification including various parameters would be to develop a nomogram (including quantitative UBC Rapid Test, grade of haematuria, smoking status, age, and gender) (26). This could be of particular interest in patients with microscopic haematuria, as the recommendations for work-up of these patients including invasive cystoscopy are discussed controversially. As the use of cell- and protein-based tests in the screening setting has shown inconclusive results (27), we could not recommend using the UBC Rapid Test in a screening population without risk factors for bladder cancer based on these preliminary.

**Conclusion**

Cystoscopy is still the most important part of monitoring of bladder cancer and it cannot yet be replaced by urinary tests. However, cytology, diagnostics of haematuria and the swift tests available at a doctor's office could in combination perhaps give a chance in the future to detect this disease earlier without a high number of cystoscopies.

From this prospective, multicentre-study we can conclude from these preliminary results that UBC Rapid can differ between patients with bladder cancer patients and healthy controls. It is very important to include a higher number of samples in this study to determine how valuable these preliminary results are.

**Acknowledgements**

The Authors would like to thank the staff of the Urological Departments at HELIOS Hospital Bad Saarow and Lukaskrankenhaus Neuss, Germany, for their excellent help while collecting the samples.

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References


Up-dated results of a multicentre-study for Urinary Bladder Cancer antigen (UBC) rapid as marker for urinary bladder cancer

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Introduction & Objectives: UBC rapid is a test detecting fragments of cytokeratins 8 and 18 in urine. They belong to the frequently overexpressed cytokeratins in tumor cells. Up to date there are no clear data for bladder cancer patients and healthy controls without disturbing factors for elevated cytokeratins in urine. We present new results of a multi-center study measuring UBC rapid in bladder cancer patients and healthy controls.

Material & Methods: Clinical urine samples were used from 195 patients with tumors of the urinary bladder (108 low grade and 87 high grade tumors) and from 136 healthy controls. Urine samples were analyzed by the UBC rapid point-of-care (POC) system and evaluated both visually and quantitatively using the concile Omega 100 POC reader. For visual evaluation, different thresholds of band intensity for considering a test positive were applied. Sensitivities and specificities were calculated by contingency analyses.

Results: We could show that pathological concentrations of UBC rapid are detectable in urine of bladder cancer patients. The calculated diagnostic sensitivity for UBC rapid in urine was 72.4% for high grade, but only 40.7% for low grade bladder cancer. The specificity was 93.4%. Pathological values of UBC rapid in urine are higher in patients with bladder cancer in comparison to the control group. The area under the curve (AUC) after receiver-operated curve (ROC) analysis was 0.738. Pathological values of UBC rapid in urine are higher in patients with bladder cancer in comparison to the control group.

Conclusions: UBC rapid can differ between bladder cancer patients and control group. Compared to published data of this cohort sensitivity for low grade tumors became higher. Further studies with a higher amount of samples will show how valuable these results are.
Initial evaluation of the diagnostic performance of the new urinary bladder cancer antigen test as a tumor marker for transitional cell carcinoma of the bladder.


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PURPOSE:
We evaluated the diagnostic performance of the new noninvasive bladder cancer test on voided urine samples from patients with transitional cell carcinoma compared to symptomatic and asymptomatic controls.

MATERIALS AND METHODS:
Urinary bladder cancer antigen was measured in urine from 86 patients with active transitional cell carcinoma of the bladder (group 1), 76 patients free of transitional cell carcinoma as confirmed by cystoscopy at followup (group 2), 25 patients with other benign urological diseases (group 3), 25 patients with other malignant pathological conditions (group 4) and 30 healthy subjects free of urological diseases (group 5).

RESULTS:
Mean urinary bladder cancer antigen concentrations were 104.84, 4.57, 11.79, 48.87 and 1.38 microg/l, for groups 1 to 5, respectively, which was statistically different (p = 0.00005) except for groups 1 and 4 (p = 0.187). Sensitivity was 87.0% (95% confidence interval 79.2 to 92.7) and specificity was 86.8% (77.1 to 93.5%), and both were optimized by receiver operating characteristics plot analysis at a threshold value of 9.74 microg/l using asymptomatic (group 2) compared to known cancer (group 1) cases.

CONCLUSIONS:
Urinary bladder cancer antigen might have a role as a potential tumor marker for diagnosing transitional cell carcinoma of the bladder.

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Abstract

We compared the ability of a new urinary bladder cancer antigen (UBC) test with conventional cytology for the detection of transitional cell carcinoma of the bladder using voided urine samples. The UBC was measured and corrected for the creatinine concentration in the urine of 61 patients with transitional cell carcinoma of the bladder (group 1), 23 patients without recurrent bladder tumors during follow-up (group 2), 28 patients with benign prostatic hyperplasia (group 3), nine patients with prostate cancer (group 4), and 90 healthy volunteers free of urological diseases (group 5). The UBC concentrations were 408.8+/-578.5, 18.8+/-26.6, 23.9+/-32.7, 17.5+/-18.6 and 4.6+/-6.7 ng/mg(-1) creatinine (mean+/-S. D.) for groups 1-5, respectively. The level for group 1 was significantly higher than for any other group. The sensitivity and specificity, which were optimized using receiver-operating characteristic curves for groups 1 and 2 were 82.0% and 82.6%, respectively, at a threshold value of 39 ng/mg(-1) creatinine. The sensitivity and specificity of cytology for these same groups were 60.7% and 86.9%, respectively. The sensitivity of the UBC was significantly higher than that of cytology, not only for total bladder tumors (82.0% vs. 60.7%, P<0.02) but also for grade I transitional cell carcinoma of the bladder (76.5% vs. 11.8%, P<0.001). While offering a similarly high specificity, the UBC test might have an advantage over cytology in terms of superior sensitivity, particularly for low-grade tumors.

Comparative analysis of sensitivity to blood in the urine for urine-based point-of-care assays (UBC rapid, NMP22 BladderChek and BTA-stat) in primary diagnosis of bladder carcinoma. Interference of blood on the results of urine-based POC tests.

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BACKGROUND:
According to guidelines, the primary diagnosis of bladder carcinoma is symptom oriented. This means that diagnostic testing is indicated for macrohaematuria, chronically recurrent microhaematuria and chronic bladder urgency. This study tests the suitability of three point of care (POC) test systems, UBC rapid, NMP22 BladderChek and BTA stat, available on the market, with respect to interference due to blood contamination in urine samples.

MATERIALS AND METHODS:

Urine samples were obtained from voluntary asymptomatic individuals without a history of bladder cancer. A specimen negative in all test systems was selected for further study. This sample was treated with fresh heparinized blood in a 1:10 ratio and then titrated in a dilution series. All the urine
samples and their consecutive test results were photographed and a urinalysis was performed on each sample.

RESULTS:

In none of the samples of the dilution series did UBC rapid or NMP22 BladderChek show a false-positive result due to blood contamination. In contrast, with the BTA stat testing system, false-positive results were obtained from all samples with macrohaematuria and with densities up to 150 erythrocytes/µl, indicating a suspected tumour, whereas the sample was actually proven to be tumour free.

CONCLUSION:

For the primary diagnosis of bladder carcinoma, neither the UBC rapid nor the NMP22 BladderChek POC test systems are sensitive to the presence of blood in the urine, whereas BTA stat consistently yields false-positive results due to cross-reactivity to macrohaematuria and microhaematuria up to a density of 150 erythrocytes/µl, thus this system should not be employed for this examination.


Comparative predictive values of urinary cytology, urinary bladder cancer antigen, CYFRA 21-1 and NMP22 for evaluating symptomatic patients at risk for bladder cancer.

Sánchez-Carbayo M¹, Urrutia M, Silva JM, Romani R, De Buitrago JM, Navajo JA.

Author information

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Abstract

PURPOSE:

We study the potential diagnostic use of urinary bladder cancer antigen, CYFRA 21-1 and NMP22*; for evaluating symptomatic patients who present with microscopic hematuria and are at risk for bladder cancer.

MATERIALS AND METHODS:

Urinary tumor markers were determined in 187 samples from 112 patients symptomatic of bladder cancer (group 1), and 75 with benign and other urological conditions (group 2). Immunoassays were used to measure the 3 selected biomarkers. Sensitivity and specificity were established by previously defined cut points. Biomarker results were reported as corrected and uncorrected for urinary creatinine. Urinalysis was performed in all samples.

RESULTS:

Positive and negative predictive values were 85.5%, 80.5% and 81.1%, and 80.8%, 79.2% and 76.5% for urinary bladder cancer antigen, CYFRA 21-1 and NMP22, with the cutoffs 9.7 microg./l., 5.4 microg./l and 10.0 units per ml., respectively. These predictives values were 85.2% and 72.5%,
respectively, for urinary cytology. The combination of biomarkers decreased the positive predictive values to 72.3% to 78.6% and increased negative predictive values to 84.2% to 86.1%. Urinary tract infection, inflammation and malignancy associated with other genitourinary organs were the primary cause for false-positive test results in the 3 assays evaluated.

CONCLUSIONS:

With a single biomarker, around 80% of the positive results would have correctly identified symptomatic patients for cystoscopy. Of the negative results 75% would have correctly reduced the number of cystoscopies. Sensitivity and negative predictive values could be improved with the combination of biomarkers but with a loss of specificity and positive predictive values. Urinary tract inflammation and other genitourinary malignancies might contribute to the reduction in specificity of these tests.


Utility of serial urinary tumor markers to individualize intervals between cystoscopies in the monitoring of patients with bladder carcinoma.

Sánchez-Carbayo M*, Urrutia M, González de Buitrago JM, Navajo JA.

Abstract

BACKGROUND:
Cross-section studies have shown the diagnostic characteristics of certain urinary tumor markers for the detection of bladder carcinoma. However, the role of serial urinary tumor markers in the monitoring of patients with bladder carcinoma in daily clinical surveillance has not been completely defined yet.

METHODS:
The study comprised 1185 urine samples belonging to 232 patients with a previous bladder carcinoma: 106 patients under follow-up (Group 1) and 126 bladder carcinoma patients receiving intravesic instillations (Group 2). Patients were monitored with urinary tumor markers during a one-year follow-up period. Urine samples were collected before cystoscopies and in the intercystoscopic periods for patients in Group 1 and before intravesic instillations for patients Group 2. Urinary bladder carcinoma antigen (UBC), CYFRA 21-1 and nuclear matrix proteins (NMP22) were measured by immunoassays.

RESULTS:
Monitoring of the disease with urinary tumor markers could detect recurrence sooner than scheduled cystoscopies in 27 patients (87%) for UBC, 27 patients (87%) for CYFRA 21-1, and 26 patients (84%) for NMP22 out of 31 Group 1 patients who recurred; and in 16 patients (67%) for UBC, 17 patients (71%) for cytokeratin fragments (CYFRA) 21-1, and 13 patients (54%) for NMP22 out of 24 Group 2 patients who recurred. The most relevant finding was that persistence of negative urinary markers during follow-up was largely indicative of disease free status in 65 of 75 (87%) patients of Group 1 and 31 of 102 (30%) cases of Group 2. Although false positive results were present, they were mainly associated with sporadic urinary tract infections in 10 of 75 (13%) cases of Group 1 and in 36 of 102
(35%) patients of Group 2; and with urine samples collected in the first two months at the beginning of intravesic therapy in 35 of 102 patients (34%) in Group 2.

**CONCLUSIONS:**
Monitoring of bladder carcinoma patients with serial urinary tumor markers could anticipate detection of recurrence. Persistent negative results might postpone and reduce the number of cystoscopies. Once the limitations leading to false positive results are controlled by urinalysis and by starting sample collection when basal levels are reached in patients with intravesic therapy, urinary tumor markers might eventually individualize the intervals between cystoscopies in the surveillance of patients with bladder carcinoma.


**Special Issue: Recent Advances in Cancer Biomarkers**

**Clinical utility of cytokeratins as tumor markers**

Vivian Barak, Helena Goike, Katja W. Panaretakis Roland Einarsson

**Abstract**

Cytokeratins, belonging to the intermediate filament (IF) protein family, are particularly useful tools in oncology diagnostics. At present, more than 20 different cytokeratins have been identified, of which cytokeratins 8, 18, and 19 are the most abundant in simple epithelial cells. Upon release from proliferating or apoptotic cells, cytokeratins provide useful markers for epithelial malignancies, distinctly reflecting ongoing cell activity. It appears that motifs in certain cytokeratins make them likely substrates for caspase degradation, and their subsequent release occurs during the intermediate events in apoptosis.

The clinical value of determining soluble cytokeratin protein fragments in body fluids lies in the early detection of recurrence and the fast assessment of the efficacy of therapy response in epithelial cell carcinomas. The three most applied cytokeratin markers used in the clinic are tissue polypeptide antigen (TPA), tissue polypeptide specific antigen (TPS), and CYFRA 21-1. TPA is a broad spectrum test that measures cytokeratins 8, 18, and 19. TPS and CYFRA 21-1 assays are more specific and measure cytokeratin 18 and cytokeratin 19, respectively. By following patients with repeated testing during management, the oncologist may obtain critical information regarding the growth activity in symptomatic patients. Although their main use is to monitor treatment and evaluate response to therapy, early prognostic information particularly on tumor progression and metastasis formation is also provided for several types of cancers. Cytokeratin tumor markers can accurately predict disease status before conventional methods and offer a simple, noninvasive, cheap, and reliable tool for more efficient management.


**Evaluation of a new quantitative point-of-care test platform for urine-based detection of bladder cancer.**

Ritter R1, Hennenlotter J1, Kühs U1, Hofmann U1, Aufderklamm S1, Blutbacher P1, Deja A1, Hohneder A1, Gerber V1, Gakis G1, Stenzl A1, Schwentner C1, Todenhöfer T2.
Abstract

OBJECTIVE:

Several commercial point-of-care (POC) tests are available for urine-based detection of bladder cancer (BC). However, these tests are restricted to dichotomized results (positive or negative), which limits their diagnostic value. Quantitative protein-based tests offer improved risk stratification but require complex methods restricted to specialized centers. Recently, the first quantitative POC system based on the detection of cytokeratin fragments became available. The aim of the study was to evaluate the diagnostic accuracy of this quantitative POC test.

PATIENTS AND METHODS:

A total of 198 patients having symptoms suspicious for BC were included. All patients received urethrocystoscopy and upper-tract imaging. Urine samples were analyzed by the urine BC antigen (UBC) rapid POC system and evaluated both visually and quantitatively using the concile Omega 100 POC reader. For visual evaluation, different thresholds of band intensity for considering a test positive were applied. Moreover, the UBC enzyme-linked immunosorbent assay (ELISA), urine cytology, and the nuclear matrix protein 22 BladderChek were performed. Sensitivities and specificities were calculated by contingency analyses. Optimal cutoffs of quantitative tests were determined by receiver operating characteristic curves.

RESULTS:

A total of 61 patients (30.8%) were diagnosed with BC. Visual evaluation of the UBC revealed sensitivities of 38.1% to 71.4% with corresponding specificities of 54.1% to 89.1%, dependent on the threshold of band intensity applied. The quantitative UBC rapid showed a sensitivity of 60.7% and a specificity of 70.1% at optimal cutoff (area under the curve = 0.68). A constant increase of both the probability of BC and high-risk BC with increasing UBC rapid values was observed. UBC concentrations determined by the reader significantly correlated with the UBC ELISA (P<0.001). The UBC ELISA, the nuclear matrix protein22 BladderChek and cytology showed sensitivities of 48.3%, 16.4%, and 51.7% with specificities of 71.3%, 95.3%, and 78.1%, respectively.

CONCLUSION:

The UBC rapid in combination with a quantitative POC-reader system for the first time enables quantitative determination of a BC marker under POC conditions. Diagnostic accuracy is at least equivalent to elaborate ELISA-based measurement. The quantitative use of the UBC rapid test facilitates risk prediction compared with conventional nonquantitative dichotomized POC testing.
Background and Aims

Urine based tumor markers have been developed as proteomics since 30 years. First they have been established as ELISA test to quantify the antigen amount in urine and to relate the marker concentration to pathological cancer expression inside of the bladder. Typical markers were BTA, NMP22, UBC and Cyfra21. In the following the parameters have been transferred to point-of-care test-systems producing qualitative results (+/-) for quick in-office results. The newest marker systems offer a point-of-care test-system with objective quantification. The first tumor marker in bladder cancer is now UBC-rapid used by a so called Omega-reader.

Our study should prove the practicability, the quality of test results in relation to controls, tumor and interfering urological illnesses to define the quality parameters like sensitivity, specificity, accuracy, NPV, PPV, the rate of false positive- and false negative test results. Furthermore we wanted to check exclusion criteria’s for the test-system to ensure accurate handling in clinical use for primary diagnosis, follow-up and possible screening in risk-populations.

Methods

We collected urine probes from defined populations separately in plastic tubes for each patient and transferred a defined volume of 1 ml to a stabilizing solution. From this volume we transferred 75 μl to the UBC-rapid test-cassette and after an incubation time of 10 minutes the so-called photometric Omega-Reader measured the antigen concentration of UBC and printed this objective result. The collected results were related to the defined populations and were used to calculate a ROC-curve, define the quality results as mentioned and to check if medical situations can be defined as exclusion criteria’s.

Results

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Results</th>
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</thead>
<tbody>
<tr>
<td>Sensitivity</td>
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<tr>
<td>Specificity</td>
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</tr>
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<tr>
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</tr>
<tr>
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<tr>
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<tr>
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</tr>
<tr>
<td>Likelihood ratio (neg)</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Inferences & Exclusion criteria’s

- UBC-rapid is biologically identical to established proteomic test-systems like NMP22 and BTA.
- In case of post-cystectomy and bowel associated urinary diversion UBC-rapid is non-useable for follow-up controls.
- UTI, catheter placement and stones inside the urinary tract are exclusion criteria for UBC-rapid.
- TCC tumor stage and grade is directly correlated to UBC concentration. Quantified results of UBC are of prognostic relevance.

Discussion

- The cytoskeletal marker UBC-Rapid is powerful in bladder cancer diagnostics.
- An interference of blood in urine with UBC-rapid can be excluded.
- The transfer to a quantifying POC-system was successful.
- The main domain for this quantitative POC-Test is the out-patient support.
  - Easy in handling  ➔ fast in result

Take home message
UBC® Rapid

- UBC® Rapid showed better performance than urine cytology due to improved sensitivity in particular for low-grade tumors.
- The combination of UBC® Rapid and cytology led to detection of additional tumors compared to cytology alone.
- UBC® Rapid measures a continuous parameter, the higher the value, the more likely bladder cancer is present.
- Quantitative use of UBC® Rapid facilitates risk prediction.

UBC® Rapid Summary and conclusion

- UBC® Rapid with the reader is a urinary Point Of Care marker providing the concentration level of Cytokeratins 8 and 18.
- UBC® Rapid is a useful indicator of what to expect cystoscopy to show.
- UBC® Rapid is a non-invasive indicator of tumour cell activity measured in urine.
- UBC® Rapid demonstrates high diagnostic sensitivity for bladder tumours.
- UBC® Rapid is a useful tool for follow-up
- UBC® Rapid concentration readings provide a useful indicator of disease progression.