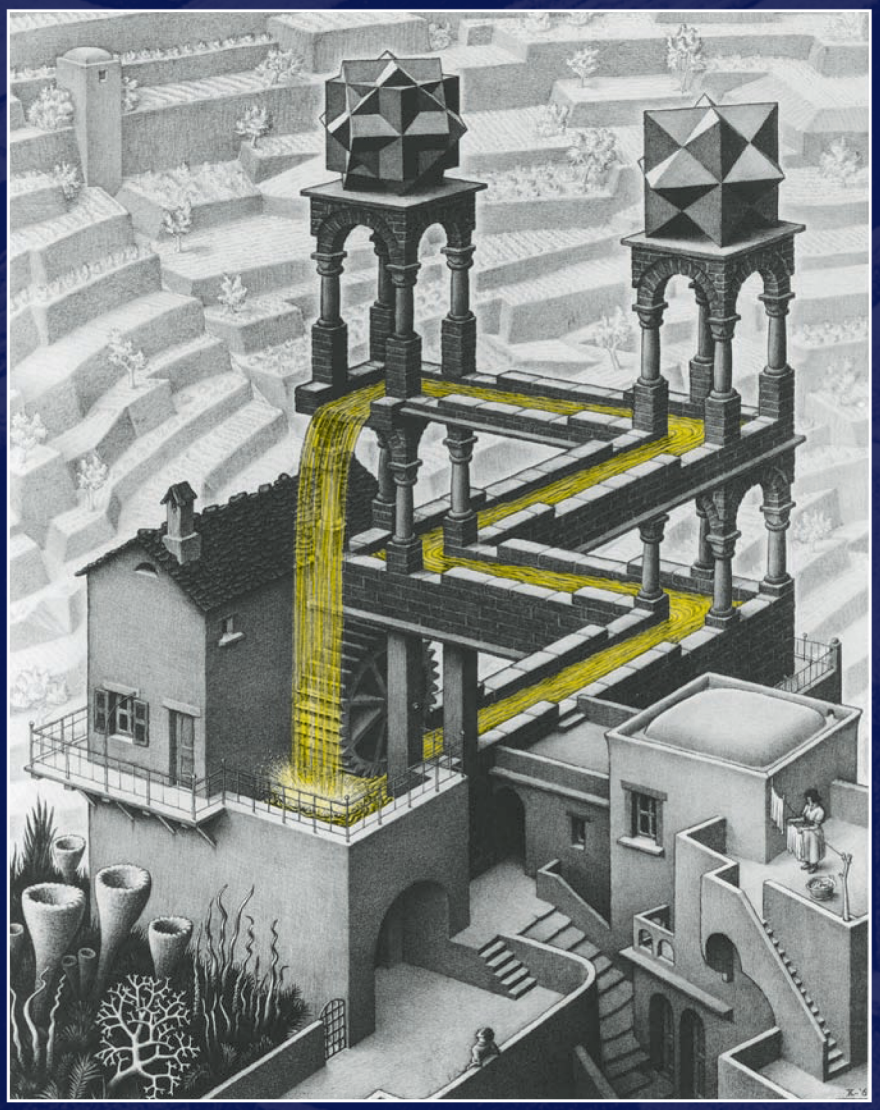
Surveillance of Bladder Cancer



Madelon van der Aa

SURVEILLANCE OF BLADDER CANCER

Madelon van der Aa
2009

The studies described in this thesis were performed at the departments of Pathology and Urology, Erasmus MC, Rotterdam, The Netherlands in cooperation with the departments of Urology and Pathology (in alphabetical order) of the Albert Schweitzer Hospitals Dordrecht, Amphia Hospitals Breda, Clara Hospital Rotterdam (MCRZ), Havenziekenhuis Rotterdam, Ikazia Hospital Rotterdam, Leids Universitair Medisch Centrum Leiden, Reinier de Graaff Groep Gasthuis Delft (SGGD), Sint Franciscus Gasthuis Rotterdam and Vlietland Hospital Schiedam.

The research was funded by ZON-MW (The Netherlands Organization for Health Research and Development), grant number 945-02-046, and the Erasmus MC Rotterdam.

Financial support for the printing of this thesis was obtained by kind contributions from: J.E. Jurriaanse Stichting, Astellas, AstraZeneca, Abbott, GlaxoSmithKline, Mathot, Nycomed, Novartis, Pfizer, Pohl Boskamp, Sanofi Aventis and Wyeth Pharmaceuticals

Cover Picture: M.C. Escher's "Waterfall" © 2008 The M.C. Escher Company B.V. -Baarn- Holland. All rights reserved. www.mcescher.com

Lay-out and cover design by Optima Grafische Communicatie, Rotterdam

Printed by Optima Grafische Communicatie, Rotterdam, www.ogc.nl

ISBN 978-90-8559-492-5

Surveillance of Bladder Cancer

Proefschrift

Ter verkrijging van de graad van doctor aan de

Erasmus Universiteit Rotterdam,

op gezag van

rector magnificus

Prof.dr. S.W.J. Lamberts

En volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op

25 februari 2009 om 9:30 uur

door

Madelon Norbertha Maria van der Aa

Geboren te Goirle

Promotiecomissie

Promotoren:	Prof. dr Th. H. van der Kwast
	Prof. dr E.W. Steyerberg
	Prof. dr E.C. Zwarthoff
Overige leden:	Prof. dr C.W. Oosterhuis
	Prof. dr C.H. Bangma
	Prof. dr M.G.M. Hunink
	Prof. J. Zwartendijk
	Prof. dr L.A.L.M. Kiemeney
	Dr W. Dinjens
Paranimfen:	Linetta B. Koppert
	Jennie Prins

Als men op een goede dag
Totaal onverwacht, oog in oog,
Met een perpetuum mobile staat

Zal men eerst schrikken
Maar dan meteen de uitdaging zien
In controle en comfort

Niets lijkt onmogelijk

Surveillance of Bladder Cancer

PART I General Introduction

Chapter 1 Background 11

Chapter 2 Outline of the thesis 63

PART II Bladder Cancer Biology

Chapter 3 Clinical and Pathological Prognostic Factors for Recurrence, Progression and Mortality in Non-muscle invasive Bladder Cancer: A Meta-analysis
Submitted. 69

Chapter 4 Prediction of Progression of Non-Muscle-Invasive Bladder Cancer by WHO 1973 and 2004 Grading and by FGFR3 Mutation Status: A Prospective Study.
European Urology, 2007. 87

Chapter 5 A new system for substaging pT1 papillary bladder cancer: a prognostic evaluation.
Human Pathology, 2005 . 103

PART III Surveillance in clinical practice

Chapter 6 Feasibility of microsatellite analysis of voided urine samples for surveillance of low-grade non-muscle invasive urothelial carcinoma, results of a prospective multicenter study (CEFUB)
European Urology, 2008. 119

Chapter 7 Cystoscopy revisited as the gold standard for detection of bladder cancer recurrences: diagnostic review bias in a randomized prospective trial (CEFuB-trial)
Submitted. 135

PART IV The Patient under surveillance / Quality of life

Chapter 8 The burden of flexible cystoscopy in patients monitored for bladder cancer 151
British Journal of Urology International, 2007.

Chapter 9 Sexual function in patients under surveillance for bladder cancer 163
British Journal of Urology International, 2008.

Appendix Questionnaires I-IV 173

PART V Public Health Effects / Economic Evaluation

Chapter 10 Cost-Effectiveness of non-muscle invasive bladder cancer surveillance in which cystoscopy is partly replaced by microsatellite analysis on urine samples 201
British Journal of Urology International, 2008.

PART VI Epilogue

Chapter 11 General Discussion, Future Perspectives & Concluding Remarks 219

Chapter 12 Summary and Samenvatting 237

Chapter 13 Acknowledgement / Dankwoord 253

Participants in CEFUB-trial 263

Contributing authors 265

List of Publications 267

PART I

INTRODUCTION

CHAPTER 1

Background

CHAPTER 2

Outline of the thesis

CHAPTER 1

Background

Table of Contents

1. Introduction
    - 1.1 Epidemiology & Etiology

2. Clinical Diagnosis
    - 2.1 Symptoms
    - 2.2 Diagnostics

3. Pathological Diagnosis
    - 3.1 Classification of bladder cancer
    - 3.2 TNM classification of bladder cancer
    - 3.3 Non-muscle invasive bladder cancer
    - 3.4 World Health Organisation (WHO) classification of bladder tumours

4. Treatment
    - 4.1 Treatment of non muscle invasive bladder cancer
    - 4.2 Treatment of muscle invasive bladder cancer

5. Biology of Bladder Cancer
    - 5.1 Genetic alterations in human cancer
    - 5.2 Genetic instability in bladder cancer
    - 5.2.1 Hereditary bladder cancer
    - 5.3 Molecular alterations in non-muscle invasive bladder cancer
    - 5.3.1 Fibroblast Growth Factor Receptor 3 gene mutation
    - 5.3.2 Chromosome 9 and loss of heterozygosity
    - 5.4 Theories concerning bladder carcinogenesis
    - 5.4.1 Molecular pathways

6. Surveillance in of bladder cancer
    - 6.1 Urethrocystoscopic surveillance
    - 6.2 Urinary markers
    - 6.2.1 Diagnostic tests
    - 6.2.2 Microsatellite analysis
    - 6.2.3 Outline of a randomized clinical trial

7. The prediction of the disease course in bladder cancer
    - 7.1 What is a clinically relevant independent risk factor?
    - 7.2 Prognostic factors

7.2.1 Clinical characteristics
7.2.2 Pathological characteristics
7.2.3 Molecular characteristics
7.2.3.1 *FGFR3 gene* mutations
7.2.3.2 TP53 tumour suppressor gene
7.2.3.3 Ki-67 antibody
7.2.3.4 CK-20 protein expression

8. Patients' opinion

9. Economic evaluation

10. Abbreviations

11. References

1. Introduction

1.1 Epidemiology & Etiology

The urinary bladder together with the pyelum, ureters and urethra form the urinary tract system (figure 1.1); the system that is responsible for the excretion and collection of urine. With approximately 357,000 new cases per year worldwide, tumours of the urinary tract system contribute significantly to the overall human cancer burden[1]. Urothelial cancer of the bladder is the fifth most common malignancy in the western world and accounts for 5% of all diagnosed cancers[2]. In Japan a much lower incidence is seen (Figure 1.2a). In the Netherlands, approximately 4,550 new cases present each year (Figure 1.2b), one of the highest incidence rates in the European Union.

The walls of the urinary tract are covered by a specialized epithelial lining, called urothelium. Approximately 90-95% of all tumours in the bladder are urothelial carcinoma (UC), previously known as transitional cell carcinomas[3, 4]. Other histological subtypes of bladder cancer are squamous cell carcinomas (< 5%), adenocarcinomas (1-2%) often originating from an embryologic remnant at the dome of the urinary bladder, i.e. the urachus, neuroendocrine tumours and soft tissue tumours (<1%), which are very rare [I]. In this thesis the general term bladder cancer will sometimes be used to refer to urothelial carcinoma (UC) although this terminology is not entirely correct.

The male-to-female ratio of UC is in most western counties approximately 3:1. Bladder cancer represents in males the fourth most common cancer, in women the eighth, both if incidence and disease specific mortality rate are considered[1]. Some studies on gender differences showed that female patients are more frequently diagnosed with higher stages of bladder cancer and upper urinary tract tumours at first presentation[5].

The origin of bladder cancer is multifactorial. In most countries cigarette smoking is by far the most important risk factor. The risk of bladder cancer in smokers is two to six fold that of non-smokers, and smoking accounts for 30-50% of all bladder cancers[6-9]. It is estimated that the risk of bladder cancer attributed to tobacco smoking is 66% in men and 30% in woman implying that women are somewhat protected against the carcinogenic effects of smoking [II]. The smoking behaviour largely explains the gender difference in incidence of bladder cancer. Other etiological factors include analgesic abuse and occupational exposure to aromatic amines. Until 1970 aniline dye in paint caused an increased frequency of bladder tumours in

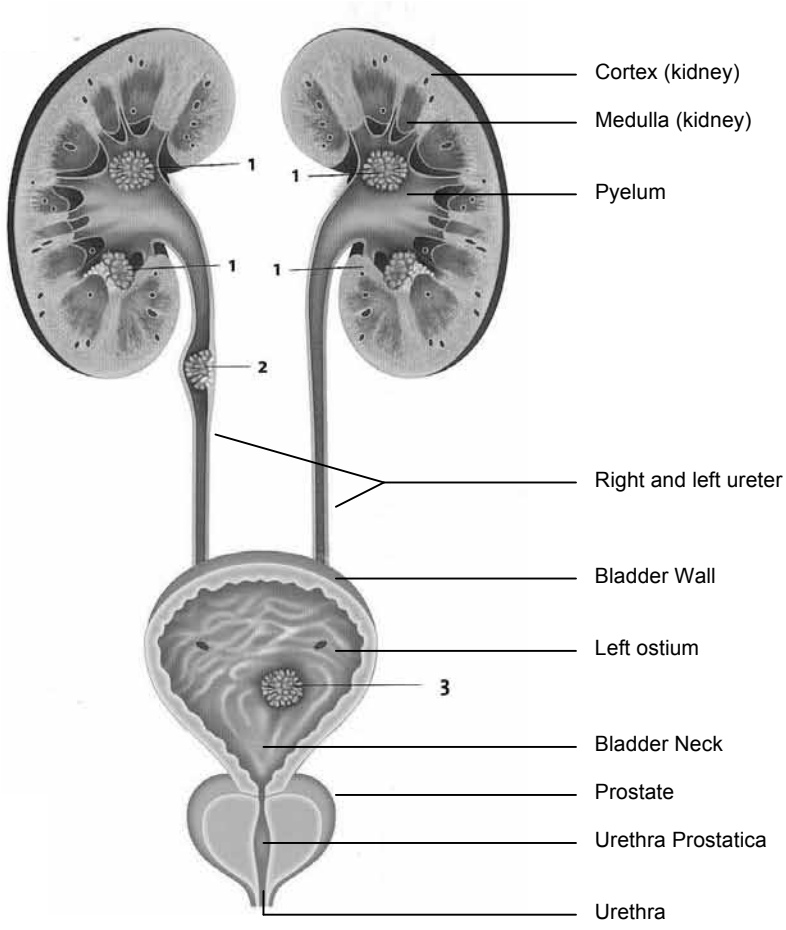
I Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB (2003). Cancer incidence in Five continents. IARC Scientific Publications No155. IARC Press: Lyon

II IARC (2004), IARC monographs on the evaluation of carcinogenic risks to humans: Tobacco smoke and involuntary smoking, IARC Press Lyon.

professional painters. In Egypt and other countries in Africa and the Middle East the endemic spread of schistosomiasis (Schistosoma haematobium) is a frequent cause of chronic Schistosoma cystitis, provoked by deposition of eggs in the tissue underlying the urothelium. As a consequence the majority of the patients with bladder cancer in Egypt are diagnosed with squamous cell carcinoma, an uncommon type of bladder cancer in the western countries where squamous cell carcinoma of the bladder has an incidence rate of less than 5%[10, 11].

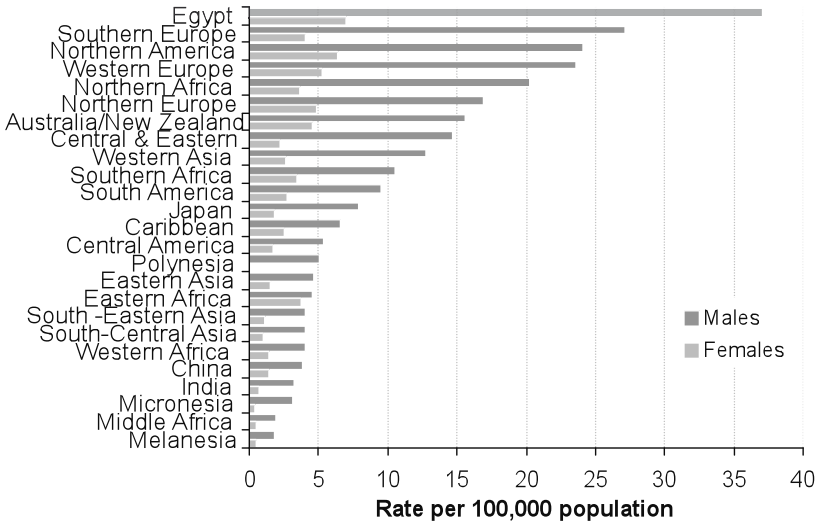
In 95% of the cases UC develops primarily in the bladder, the other 5% develops in the pelvicalyceal system, ureter or urethra. Tumours of the ureter and renal pelvis account for 8% of all urinary tract neoplasms and of these more than 90% are urothelial carcinomas[12]. The

Figure 1.1



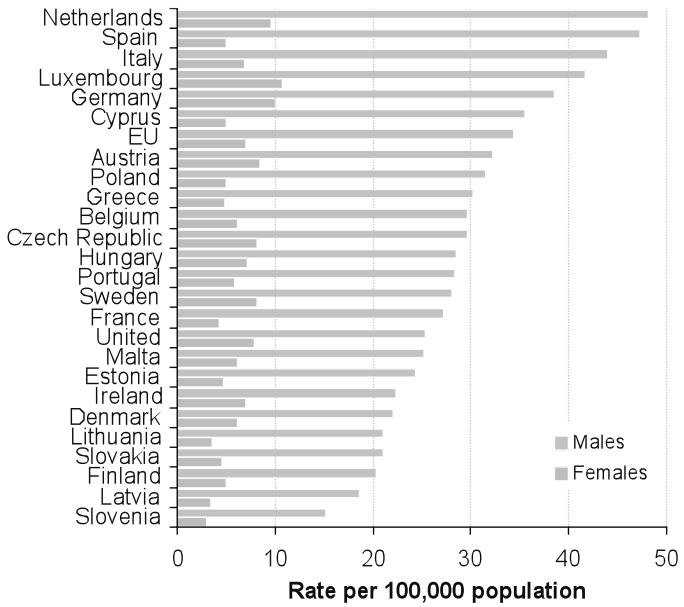
Urinary tract system: urothelial cancer originates from the urothelial lining of the pyelo-caliceal system (pyelum), ureters, the bladder and urethra. 1 papillary tumours in the pyelo calyceal system; 2 papillary tumours in ureter; 3 papillary tumours in the bladder.

Figure 1.2a



Age-standardized (world) incidence rates for bladder cancer, by sex and world regions, (estimates over 2002). Source: Cancer Research UK, bladder cancer (www.cancerresearchuk.org).

Figure 1.2b



Age-standardized (European) incidence rates for bladder cancer, by sex and EU countries, (estimates over 2002). Source: Cancer Research UK, bladder cancer (www.cancerresearchuk.org).

incidence of these tumours had increased slightly in the past 30 years. Compared to UC of the bladder, there is a different male to female ratio of 1.7 to 1 with an increasing incidence in females[13].

The renal pelvis is together with the ureter, bladder and urethra, part of the lower urinary tract. Nevertheless clinicians generally refer to the UC of the renal pelvis and ureter as upper urinary tract tumours. Malignant tumours of the renal pelvis are twice as common as those of the ureter[14] and they are frequently associated with additional UC in the urinary bladder and contralateral pelvis and ureter[15]. In 80% of the cases the malignant tumours that arise in the pyelum or ureter follow an earlier diagnosis of urinary bladder UC[16] and in 65% of the cases urothelial tumours develop at multiple sites in the urinary tract[17].

The following paragraphs will first discuss the symptoms of patients with UC, the pathological diagnosis with corresponding clinical management and the biological characteristics and subsequently we will focus on the surveillance and prognosis of urothelial carcinoma.

2. Clinical Diagnosis

2.1 Symptoms

Bladder cancer is accompanied by a few symptoms that may alert patients that something is wrong. The most important and often first symptom patients present with is painless haematuria. In 85% of the patients the haematuria will only be microscopically visible, the other 15% of the patients will present with red coloured urine. In approximately 25% of the patients irritative bladder symptoms are the first symptom; e.g. dysuria (painful and difficult voiding), stranguria (burning sensation during voiding), pollakisuria (frequent voiding), painful bladder contractions after voiding, infections or symptoms of urethral obstruction. In case the tumour process obstructs the ureter, the accumulation of urine will compress the renal parenchyma (hydronephrosis) and cause pain in the kidney region. Patients with a flat cancerous lesion of the bladder mucosa (carcinoma in situ (CIS)) may present with dysuria but are often asymptomatic. The diagnosis of bladder cancer may be delayed if the patient misinterprets the complaints of intermittent bleeding to urinary tract infection, kidney or bladder stones or menstrual bleeding (patient delay). Physicians may also misinterpret the symptoms as cystitis or other lower urinary tract problems (doctors delay).

2.2 Diagnostics

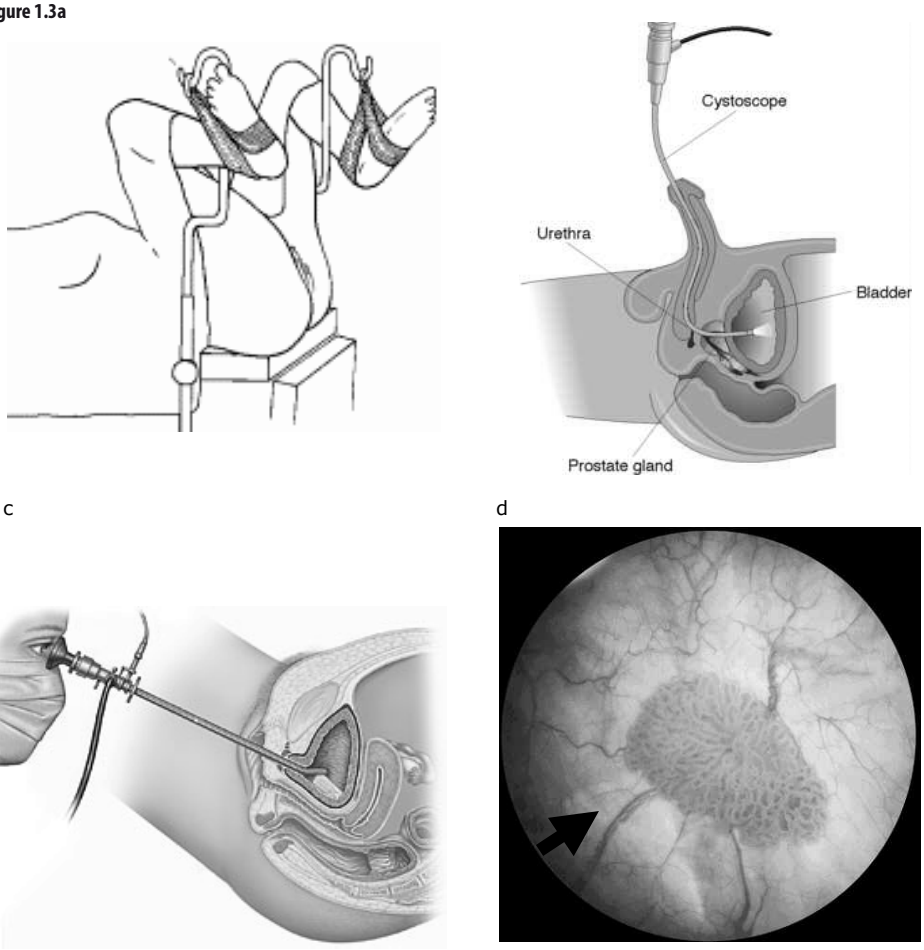
Once the patient presents at the outpatient clinic the urologist will take a history of the patient, his or her urine will be examined by cytology and, if available, a urine test will be performed in order to further evaluate the possibility of a bladder cancer. Bladder cancers will usually present as polypoid masses protruding in the lumen of the bladder. This makes

them easily visible by endoscopic inspection (cystoscopy). Thus, if clinical suspicion is high enough a cystoscopy will be performed. This technique however may not easily detect the less frequent flat bladder cancers like carcinoma in situ. To determine whether UC is present in the pyelo-caliceal system or ureter, the patient will undergo radiological imaging examinations as ultrasound, intravenous urography (IVU) or retrograde pyelogram (RPG), and CT (computed tomography)-urography (currently proposed as the standard in patients older than 50 years with macroscopic haematuria).

Urinary cytology is performed on the sediment of freshly voided urine samples. Both normal urothelial cells and tumour cells are exfoliated into the urine and these cells shed in the urine are microscopically examined. For this purpose, voided urine is centrifuged; the sediment is spread on a glass slide and stained with the Papanicolaou technique. Cytology is highly specific (86%), especially for high-grade UC and CIS (see pathology), but has a low sensitivity for low-grade UC (58%). As a consequence, when urinary cytology is positive a high-grade UC is very likely. A positive urinary cytology may indicate the presence of a UC anywhere in the urinary tract, including the renal pelvis, the ureters, the urinary bladder or urethra. A negative cytology result occurs in approximately half of the patients with bladder cancer and does not exclude the presence of a low-grade bladder cancer. Many studies evaluated the use of urinary markers for the diagnosis of UC in patients under clinical suspicion for UC in the urinary tract. Tests for bladder tumour antigen (BTA), nuclear matrix protein 22 (NMP 22), fibrin-degradation products, Quanticyt, Immunocyt and multi-targeted fluorescence in situ hybridisation (FISH) in voided urine have been introduced in the clinical setting. However none of these tests are implemented in routine clinical practice, partly due to their lack of accuracy. Although most of these diagnostic tests have a better sensitivity than cytology their specificity is lower.

Cystoscopy is an endoscopically based technique that uses the entrance via the urethra to examine the bladder and therefore this procedure is also refered to as urethro-cystoscopic surveillance (UCS) (Figure 1.3). During the procedure patients are in lithotomy position so optimal manual examination of the pelvic area is also possible. Looking inside the hollow organs and body cavities of human beings is a concept advocated in 1806 by Philipp Bozzini. Already in 1877 Maximilian Carl-Friedrich Nitze (1848-1906) used the first cystoscope in Vienna. A growing interest in kidney and bladder diseases made him realize that a successful treatment depends on an accurate diagnosis[18]. Since then cystoscopy has become the mainstay in the diagnosis of urinary bladder disease with a high sensitivity and specificity for the detection of the most common polypoid or papillary lesions[19-21]. Flat lesions are more difficult to detect and recent studies have aimed to improve their visibility by cystoscopy (note: sensitivity of cystoscopy) by using photodynamic diagnosis (PDD). Photosensitizers proved their use in enhancing the visual demarcation between normal and neoplastic tissue[22]. Fluorescence or

Figure 1.3a



Lithotomy position allows optimal examination of the bladder and pelvic floor by (a) rigid (c) and flexible (b) cystoscope in females (c) and males (b). Endoscopic view of a papillary lesion of the urothelium in the bladder (d).
Rigid and flexible scope (lithotomy position)

photodynamic cystoscopy is performed using blue light and a porphyrin-based photosensitizer, (Hexi)-aminolaevulinic acid (HAL or ALA). Because HAL has higher bioavailability than ALA and is distributed throughout all urothelial layers, HAL is preferred. It has now been demonstrated that HAL is better able to detect papillary tumours, dysplasia and CIS[22]. However promising, this investigational method has not yet been implemented on a regular basis in daily practice. This is partly due to the extended time that patients need to be prepared at outpatient clinic and the costs related to the specially needed instrumentation. Current practice is to instill a photosensitizer in the bladder during 1 hour and subsequently perform the cystoscopic examination within 1 hour after emptying the bladder. Several clinical studies analysed the accuracy of classical white light cystoscopy (until now the gold standard) by comparing with photodynamic diagnosis (PDD). In these studies the sensitivity of white light

cystoscopy for detection of any bladder cancers, including flat lesions, was demonstrated to range between 68-86%[23-30]. In this thesis we will also critically assess the sensitivity of white light cystoscopy, in the light of our surprising findings in a randomized trial conducted on a large number of patients with low risk bladder cancer.

3. Pathological Diagnosis

3.1 Classification of bladder cancer

The aim of classifying tumours is to delineate subsets of tumours, which are similar in clinical behaviour and treatment response. Clinical and histopathological criteria are generally the basis of such a tumour classification system. Owing to the progression in molecular pathology research 1) more and more genetic and biologic differences between tumours are identified and 2) the current clinical and histological classifications may in the future be supplemented

Table 1.1 TNM Classification of urinary bladder cancer, UICC 2002

T – Primary Tumor	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma in situ: "flat tumour"
T1	Tumour invades subepithelial connective tissue
T2	Tumour invades muscle (m detrusor)
T2a	Tumour invades superficial muscle (inner half)
T2b	Tumour invades deep muscle (outer half)
T3	Tumour invades perivesical tissue
T3a	Microscopically
T3b	Macroscopically (extravesical mass)
T4	Tumour invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall
T4a	Tumour invades prostate, uterus or vagina
T4b	Tumour invades pelvic wall or abdominal wall
N – Regional Lymph Nodes	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single lymph node 2cm or less in greatest dimension
N2	Metastasis in a single lymph node more than 2cm but not more than 5cm in greatest dimension, or multiple lymph nodes, one more than 5cm in greatest dimension
N3	Metastasis in a lymph node more than 5cm in greatest dimension
M – Distant Metastasis	
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

Table 1.2 Stage grouping according to TNM Classification of urinary bladder cancer, UICC 2002

TNM Classification Stage	T	N	M
0a	Ta	N0	M0
0is	Tis	N0	M0
I	T1	N0	M0
II	T2a,b	N0	M0
III	T3a,b	N0	M0
	T4a	N0	M0
IV	T4b	N0	M0
	Any T	N1, N2, N3	M0
	Any T	Any N	M1

Table 1.3 WHO grading 1973 and 2004

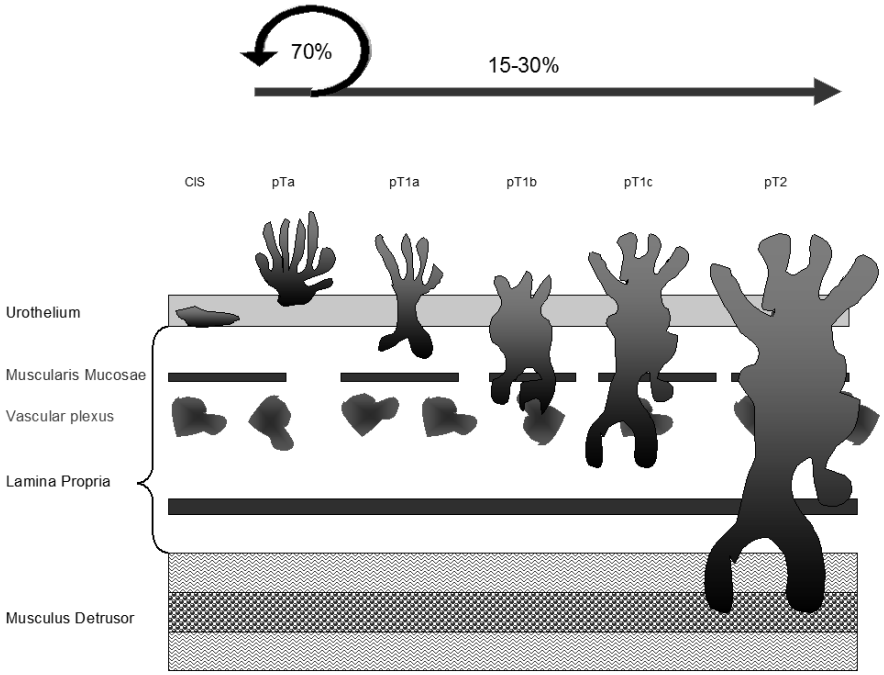
1973 WHO grading
○ Urothelial papilloma
○ Grade 1: well differentiated
○ Grade 2: moderately differentiated
○ Grade 3: poorly differentiated
2004 WHO grading
○ Urothelial papilloma
○ Papillary urothelial neoplasm of low malignant potential (PUNLMP)
○ Low-grade papillary urothelial carcinoma
○ High-grade papillary urothelial carcinoma

by molecular genetic parameters. Current treatment is still mainly based on the following two histopathological parameters: 1) tumour extension (staging) according to the most recent TNM classification of 2002 (Table 1.1 and 1.2) and 2) the differentiation grade of the tumour, determined by a process called tumour grading (according to the WHO-ISUP classification most recently adjusted in 2004, Table 1.3). Specifically tumour grading is an important task of the pathologist.

3.2 TNM classification of bladder cancer

The TNM classification defines the extension of invasion of the primary tumour (T), the metastatic spread into local or regional lymph nodes (N) and the presence of metastases in organs at distance (M). The Union International Contre le Cancer (UICC) approved the current classification in 2002 (Figure 1.4). If the clinical staging performed by clinician and radiodiagnost (cTNM) has been further confirmed by histopathological examination of tissues surgically removed from the patient the suffix "p" is added to the stage (pTNM).

Figure 1.4



Stage classification of bladder cancer according to TNM classification of 2002, but also including the most commonly applied subdivision of pT1 bladder cancers (i.e. pT1a, pT1b and pT1c); CIS (high grade intra-urothelial neoplasia), pTa (non-invasive papillary urothelial carcinoma), pT1 (invasion of urothelial carcinoma into the lamina propria). pT1a: invasion above MM and VP, pT1b: invasion into MM and/or VP and pT1c: invasion beyond VP, pT2: invasion into the muscular detrusor). MM: muscular mucosae, VP: vascular plexus, LP: lamina Propria, CIS: Carcinoma in situ.

T category

To define the extension of invasion of a bladder tumour, cystoscopic examination provides a limited role[18, 31, 32]. Transurethral resection of all visible lesions down to the deepest parts of the lesions is required for an accurate histopathological assessment of depth of tumour invasion. A complete and correct TUR is essential to determine the prognosis of the patient.

A non-invasive papillary UC that is histologically classified as pTa is limited to the bladder mucosa, whereas a tumour, which invades the subepithelial connective tissue, but not the deep muscle, is classified as pT1. The distinction between pTa and pT1 UC can only be made by histopathological examination of the resected bladder tumour fragments. An accurate pathologic staging of the tumour is very important since the treatment of a pTa/T1 high-grade and a pT2 tumour is completely different. It should be kept in mind that there is a 10% risk of pathological understaging of the tumour[33].

The (non-invasive) pTa and superficially invasive (pT1) or non muscle invasive (NMI) UC have been collectively termed by clinicians as "superficial bladder cancer". For the subset of

papillary pT1 UC and CIS, the clinical management is not standardized. This is a consequence of the elusive biologic behaviour of individual cases[34, 35]. Clinicians are inclined to monitor and treat pT1-tumours, in particular the ones with high-grade in combination with carcinoma in situ (CIS), more aggressively, because their much higher risk of progression to muscle invasive UC[36, 37]. Clinically muscle invasive tumours (≥T2) can be distinguished from non-muscle invasive UC (Ta, T1) by bimanual examination (vaginal or rectal) which can also reveal large muscle infiltrating tumours (≥T2). In cystectomy specimens, but not in transurethral resections of bladder tumours the pathologist can determine if and to what extent the tumour cells have invaded the muscularis propria: superficial (pT2a) or deep (pT2b). Stage T3 tumours extend either microscopically (pT3a) or macroscopically (pT3b) in the perivesical fat. Stage T4 tumours extend into contiguous organs, like colon, prostate, uterus or pelvic wall. The accuracy of imaging techniques (CT, MRI, PET) for determining the T-category is limited[38-40].

N category

The N-stage is determined by the assessment of regional lymph nodes for the presence of lymph-borne metastatic disease. Numerous studies investigated the impact of CT and MRI for the detection of regional lymph node metastases, but their sensitivity remains limited (33%)[41-43]. Nevertheless, lymph node enlargement (> 1 cm) on CT or MRI is highly predictive of metastatic disease[44].

M category

The presence of blood borne distant metastasis of bladder carcinomas (M-stage) can be determined by an X-ray of the lungs and imaging of the liver (ultrasound, CT, MRI). Skeletal scintigraphy for the detection of bone metastases should be performed in symptomatic patients. About 50% of patients with muscle-invasive bladder cancer already have occult distant metastases at the time of diagnosis, which limits the efficacy of local therapy.

The 5-year survival with surgery (radical cystectomy) alone is about 45% in patients with pT2N0M0 and approximately 35% in patients with pT3N0M0 bladder cancer. Patients with advanced disease, that is with positive lymph nodes (N+) and or distant metastases (M+) have a 5-year survival rate of only 10%.

3.3 Non-muscle invasive bladder cancers

("Superficial" bladder cancers)

Fortunately, 80% of the patients first presenting with symptoms of bladder cancer is diagnosed with primary NMI UC. Until recently these NMI UC were grouped under the heading "superficial bladder cancer" because of their similar therapeutic approach; both can be radically removed by transurethral resection. However, clinical experience has demonstrated

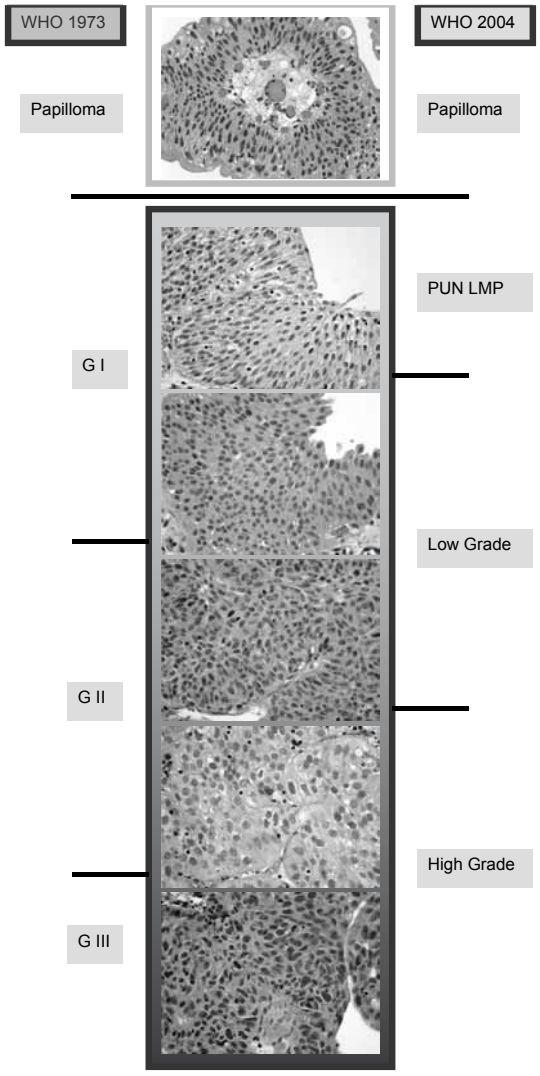
the highly unpredictable behaviour of pT1 lesions and from a molecular biological perspective they often share genetic alterations with the unfavourable muscle invasive UC (pT2). For this reason the term "superficial bladder cancer" is currently considered obsolete. The clinical course of both pTa and pT1 papillary urothelial carcinoma (UC), now also commonly described as non-muscle invasive UC, is characterized by a high tendency to recur (up to 70%) and a propensity to progress in grade (10-30%) or stage (10-15%)[2]. The follow-up policy and treatment of these patients is predominantly dictated by conventional parameters like grade and the associated presence of flat lesions like carcinoma in situ (CIS)[45, 46]. Urothelial carcinoma in situ (CIS) is a non-invasive, flat lesion (lacking papillary formations) in which the surface epithelial lining contains cells that are cytologically malignant. The intra-urothelial CIS accounts in the solitary form for less than 1-3% of urothelial neoplasms. However (secondary) CIS is present in 7-15% of papillary neoplasms and concomitant with muscle invasive UC in 45-65%[47-51]. Given the highly variable behaviour of pT1 UC an attempt was made to stratify pT1 UC on the basis of the depth of invasion of the lamina propria by a micrometer[34], or by the assessment of invasion relative to the muscularis mucosa and venous plexus[52, 53]. Although this substaging of pT1 UC was reported to have prognostic significance, the recognition of the depth of invasion by morphological criteria may be problematic because of tangential sectioning, thermal and mechanical injury or inverted (pushing) growth[34, 52, 54, 55].

3.4 WHO classification of bladder tumours

Histopathological criteria are essential to differentiate benign and malignant urothelial neoplasms. These histopathological criteria comprise architectural patterns and features of urothelial differentiation. The differentiation of urothelial cells refers to the extent to which they resemble the healthy urothelium, both morphologically and functionally. Well-differentiated cells resemble very closely their normal counterparts. The more malignant neoplasms are characterised by an increasing lack of differentiation; loss in uniformity of individual cells and loss of architectural orientation, pleomorphism (marked variation of cells in shape and size), hyperchromatic and large nucleoli, increased nuclear-cytoplasmic ratio and presence of (atypical) mitoses.

In 1973 the WHO made a first attempt to develop a worldwide applicable bladder tumour classification. The aim was to offer a standard of histopathological criteria to distinguish subsets of bladder cancers and to promote a uniform bladder tumour terminology. Most importantly, the classification system needed to be sufficiently reproducible and comprehensive to be applied by all pathologists and urologists. The grading system of the WHO 1973 bladder tumour classification recognized three different degrees: G1 (well differentiated), G2 (moderately differentiated) and G3 (poorly differentiated). Grade 3 carcinoma is related to a worse prognosis than grade 1 or grade 2 carcinoma, which is supported by several studies[56]. Grade 3 UC is strongly associated with genetic instability, concomitant carcinoma in situ (CIS), and muscle-invasive UC. However, two issues regarding the WHO 1973 classification

Figure 1.5



Comparison of the World Health Organization (WHO) Classification Systems 1973 and 2004. The vertical column represents the continuum of grades of urothelial neoplasms from the lowest (at the top) to the highest grade (at the bottom). The transverse lines at the left side of the bar illustrate the cut-offs as indicated by the WHO 1973 grading system and the transverse lines at the right side of the bar the cut-offs along the same spectrum for the WHO 2004 classification. The photomicrographs (x40) illustrate from top to bottom (from order to disorder and increase in variation); PUN LMP-Grade 1 (papillary urothelial neoplasm of low malignant potential), low-grade-Grade 1 (LG GI), low-grade-Grade II (LG GII), high-grade-Grade II (HG GII) and high-grade-Grade III (HG GIII).

system prompted its revision: 1) the terminology of "carcinoma" for the subset of non-invasive papillary UC with indolent behaviour, 2) the lack of well-defined criteria for each of the three grades, resulting into a high inter-observer variation and into disproportionally large number of grade 2 cancers. In the WHO 1973 bladder tumour classification the grade 2 cancers were

mainly defined as cancers, which did neither fulfil the criteria of grade 1 nor those of grade 3 carcinomas ("garbage-bin" function of grade 2).

The WHO 2004 classification system of bladder neoplasms distinguishes flat lesions, non-invasive and invasive papillary lesions. Flat lesions include hyperplasia, dysplasia and carcinoma in situ, while non-invasive papillary lesions may range from the benign urothelial papilloma, to borderline malignant papillary urothelial neoplasm of unknown malignant potential (PUN-LMP) and low and high-grade non-invasive (pTa) urothelial carcinomas (Figure 1.5a and 1.5b). PUNLMP is a papillary urothelial tumour that resembles the benign papilloma with regard to its cytonuclear features (lacking atypia) but shows an increased number of all layers of the urothelium. Its prognosis is excellent, progression to invasive carcinoma is rare, but recurrences may occur in 25-35% of patients.
Non-invasive papillary (pTa) low-grade UC shows contrary to PUNLMP variations in nuclear features such as size, shape and chromatin pattern. Low-grade UC shows an orderly appearance of papillary stalks with increased mitotic activity, but high-grade non-invasive papillary UC conversely shows a predominant pattern of disorder with large variations in nuclear features, prominent nucleoli and frequent mitoses. Low-grade non-invasive (pTa) UC progresses to invasive disease in less than 5% of cases, but recurrences are common, i.e. 48-71% in the first year after diagnosis[57-59]. High-grade UC is associated with poorer prognosis, as most pT1 and muscle invasive UC are high-grade. In the WHO 2004 terminology, carcinoma in Situ (CIS), a non-papillary flat lesion hiding within the surface epithelium, is also labelled high-grade intra-urothelial neoplasia. (Primary or isolated) CIS accounts for less than 1-3% of urothelial neoplasm, but (secondary) CIS is concomitantly seen in 45-65% of infiltrating UC and in 7-15% of papillary neoplasms. Data suggest that de novo (primary) CIS is less likely to progress to invasive disease than secondary CIS[60-63].

4. Treatment of bladder cancer

4.1 Treatment of non-muscle invasive bladder cancer

Primary complete transurethral resection (TUR) is the recommended treatment of both pTa and pT1 UC. A second resection, re-TUR, should be performed in high-grade tumours or if the initial resection has been incomplete. The presence of residual tumour at the site of initial resection is frequently observed during control cystoscopy at three months after TUR. The high recurrence rate at 3 months after TUR indicates that TUR is incomplete or provokes recurrences in a considerable percentage of patients[46]. It is therefore necessary to give every patient adjuvant therapy, which is intravesical chemotherapy within 24 hours after TUR, decreasing the relative risk of recurrence by 40%. Mitomycine C, epirubicine and doxirubicine have all shown to provide an equally beneficial effect. A meta-analysis demonstrated that one

post-operative instillation would prevent on average 12 TURs in every 100 patients, which is highly cost-effective[64].

The need for further intravesical therapy during follow-up in the year after TUR depends on the patient's risk of recurrence and/or progression. Patients with multiple tumours, large tumours (≥3 cm) and highly recurrent tumours (>1 recurrence/year) are at the highest risk of recurrence while patients with stage pT1 tumours, high-grade tumours and CIS have the highest risk of progression. The choice between intravesical chemotherapy (Mitomycine C, epirubicine, doxirubicine) and intravesical immunotherapy (BCG; bacillus Calmette-Guerin) largely depends on the risk that needs to be reduced: recurrence or progression. Adjuvant chemotherapy administered locally by bladder instillation is effective in preventing recurrence in low-grade UC and is associated with minor side effects. For high-grade UC and for CIS, BCG immunotherapy, consisting of induction and maintenance regimens, has proven to be superior to intravesical chemotherapy[65, 66]. BCG is a very effective treatment, but due to its substantial risk of toxic side effects only the high-risk tumours (multiple pT1 low-grade, pTa-T1 high-grade with or without concomitant CIS, CIS alone) should be treated with BCG. Nonetheless, in more than 10-15% of the patients with high-risk tumours the tumour will progress in spite of the intravesical therapy. Cystectomy may be indicated for high-risk non-muscle invasive (pT1 high-grade and BCG-resistant CIS) and for extensive papillary disease that cannot be controlled with conservative measures. In all patients with pT1 UC who fail intravesical therapy cystectomy is considered a good option. Delay in cystectomy increases the risk of progression and cancer-specific death.

4.2 Treatment of muscle invasive bladder cancer

Cystectomy is the preferred curative treatment for localised bladder neoplasm; T2-T4a, N0-Nx, M0 and high-risk non-muscle invasive UC. Radical cystectomy includes removal of the bladder, together with prostate and vesiculae seminales (in male) and together with uterus, both adnex and part of the ventral site of the vagina (in females), and removal of regional lymph nodes of which the extent of dissection has not been sufficiently defined[67-69]. According to some authors pelvic lymph node dissection up to the aortic bifurcation represents the state-of-art procedure, a potential therapeutic impact has been attributed to this procedure[69-71]. The urethra will be radically removed if there is evidence of CIS and/or UC in the bladder neck or prostatic urethra. Before cystectomy, the patient should be counselled adequately regarding all possible alternatives in urinary diversion techniques and post-operative care.

Three different techniques for a urinary diversion are available. Most commonly is the uretero-ileocutaneostomy according to Bricker, which is an incontinent stoma demanding a permanent reservoir for the collection of urine. The second possible diversion procedure is a continent stoma called 'Indiana pouch' surgically shaped from the ileo-coecal corner, which needs to be emptied by self-catheterisation through a passage in the belly button or abdomen. A third form of diversion is an orthotopic bladder substitution or neobladder.

In this latter type an isolated part of the ileum forms a reservoir that is directly connected to the urethra. Contraindications for orthotopic bladder substitution are UC and/or CIS of the prostatic urethra or bladder neck (females), high-dose preoperative irradiation, complex urethral stricture and pre-existing incontinence. The terminal ileum and colon are the intestinal segments of choice for urinary diversion. The type of urinary diversion does not affect oncological outcome[72-74]. Mortality as a consequence of the cystectomy in combination with a urinary diversion is less than 2%.

Preoperative radiotherapy does not show any survival benefit[75]. Neoadjuvant cisplatin-containing combination chemotherapy improves overall survival by 5-7% at 5-years, irrespective of the type of definitive treatment[76].

5. Biology of Bladder Cancer

5.1 Genetic alterations in human cancer

Cancer is a disease of the genome with accumulation of DNA alterations in premalignant tissue, leading to evolution of cell clones with increasing genomic instability and finally to the development of cells with invasive and metastatic capabilities[77]. Most mutations found in human malignancy are somatic and found only in the patient's tumour tissue. It is generally accepted that some of these alterations are causally involved in the transition of a normal cell into a tumour cell[78]. Additionally, epigenetic alterations (e.g. methylation of DNA sequences resulting in gene silencing) can play a role in the cellular transformation. The development of cancer is a multistep process of genetic and epigenetic alterations. These alterations lead to dysregulation of proliferation, apoptosis, and may cause an epithelial-mesenchymal transition underlying invasion and ultimately metastasis.

Most tumours are characterised by increasing genomic instability further facilitating the accumulation of mutations[79]. The variations caused by chromosome abnormalities include numerical and structural changes. Numerical changes of the genome result in a deviation of the normal set of 46 chromosomes. Aberrations in chromosome structure are caused by breakage and reunion of chromosome segments during mitosis and by environmental influence of agents like radiation, chemicals or viruses. Genetic instability exists at two levels[80]; microsatellite instability and chromosomal instability (CIN). Microsatellite instability is defined as alterations in the length of stretches of di, tri or tetra nucleotide repeats, and is generally the consequence of defects in the mismatch DNA repair system. Chromosomal instability reflects the alterations at the chromosomal level, as manifested by losses of smaller or larger chromosomal regions or even entire chromosomes. The targets of the genomic instability, at the nucleotide or at the chromosomal level, comprise 2 classes of genes[81]: proto-oncogenes and tumour suppressor genes. The activation of proto-oncogenes and inactivation of tumour suppressor genes as the result of genetic instability form the key contributions to tumour de-

velopment. Excessive cell growth can result from either oncogene activation or inactivation of a tumour suppressor gene. To date no proto-oncogenes or tumour suppressor genes have been found that are indiscriminately activated or inactivated in all cancers. Even comparable cancers from the same organ and cell type never completely share alterations in the same genes. However comparable tumours might probably have alterations in the same molecular pathways but with different components affected[82-84].

5.2 Genetic instability in bladder cancer

Microsatellite instability is found in a very small subset of bladder cancers, and these tumours are predominantly diploid. In most bladder cancers instability is observed at the chromosomal level (CIN), resulting in losses and gains of partial or even entire chromosomes (aneuploidy)[85]. The contribution of CIN is mostly minor in NMI UC compared to muscle invasive UC were CIN is frequently displayed.

5.2.1 Hereditary bladder cancer

Genes mutated in the germ cells can cause hereditary cancer syndromes such as the *BRCA1*, *BRCA2*, *CHEK2* and *TP53* gene mutations associated with breast cancer, *APC* gene mutations associated with colonic cancer and mutations in mismatch repair genes associated with hereditary non-polyposis colon cancer. In the latter families an increased frequency of upper urinary tract UC (renal pelvis, ureter) and bladder is observed[86-91]. Extended families with bladder cancer are very rare. A recent study with high-resolution array based CGH (Comparative Genomic Hybridization) in 10 high-risk UC families in The Netherlands showed no evidence of candidate regions for a gene that may predispose to the development of bladder cancer[92]. However, very recently it was demonstrated that germ line mutations in the CHEK2 kinase gene are associated with a slightly (OR 1.9) increased risk of bladder cancer[93].

5.3 Molecular alterations in non-muscle invasive bladder cancer

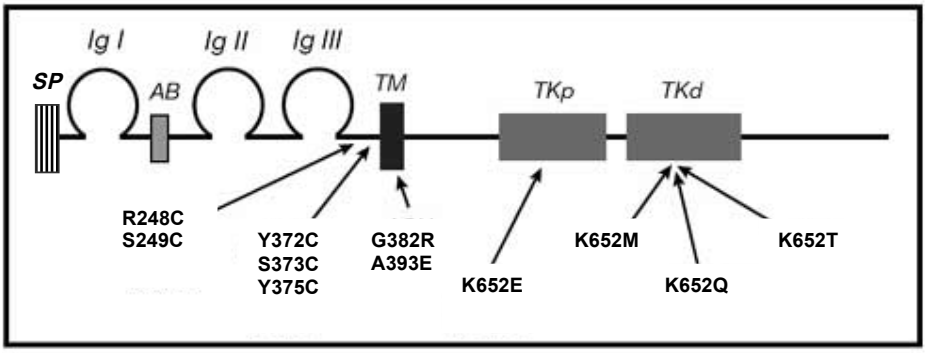
Many studies have assessed the status of known oncogenes and tumour suppressor genes and have searched for common chromosomal alterations to identify novel genomic events in bladder cancer[94-97].

Non-muscle invasive UC displays few molecular alterations. Comparative genomic Hybridisation (CGH) has identified different copy number changes including gain of 1q, 17 and 20q, amplifications of 11q and loss of 10q but none of these are as frequent as the deletions involving chromosome 9. Loss of heterozygosity analysis (LOH) has revealed frequent loss of 9q (60% concerning all UC), 4p (22% all UC), 8p (23% in high-grade, high stage UC), 11p (40% correlated with tumour grade), and 17p (p53 loci, associated with muscle invasive UC). Next to the genetic alterations involving chromosome 9 are the mutations of the Fibroblast Growth Factor receptor 3 (FGFR3) representing the most frequently found molecular alteration in non-muscle invasive UC.

5.3.1 The Fibroblast Growth Factor Receptor 3 gene

One of the most exciting recent discoveries in bladder cancer molecular genetics is the frequent observation of gain of function mutations in the *Fibroblast Growth Factor Receptor 3* (*FGFR3*) gene[98-104] (Figure 1.6). The *FGFR3* gene is located on chromosome region 4p16.3[105]. Abnormal activation of FGFR3 can be caused by translocation of the gene region to chromosome 14 leading to overexpression or by activating point mutations in the *FGFR3* gene. Activating mutations of FGFR3 are found in the germline in several autosomal dominant human skeletal syndromes associated with dwarfism, like achondroplasia, hypochondroplasia, severe achondroplasia with developmental delay and acanthosis nigricans (SADDAN),

Figure 1.6



Schematic picture of the FGFR3 protein consisting of an *extracellular* domain: SP (NH_2-terminal hydrophobic Signal Peptide), an Ig (three immunoglobulin-like domains (Ig I, II and III)), an AB (acid box); a *transmembrane* domain (TM) and an *intracellular* domain TK (tyrosine kinase domain). Mutations may occur in all three domains, the arrows point to the location of the eleven *FGFR3* mutations found in bladder cancer.

thanatophoric dysplasia types I and II (TD) and Crouzon Syndrome. In the germline, activating mutations have profound effects on the growth of the long bones where premature differentiation of chondrocytes in the growth plates is induced, causing shortness of stature (Figure 1.7). Besides the inhibitory role of FGFR3 mutations in skeletal disorders, an oncogenic role for FGFR3 in human cancer has emerged. In somatic cells translocations of the FGFR3 gene region are found in Multiple Myeloma (MM). Overexpression leads to myeloma cell proliferation and prevents apoptosis[106]. Activating mutations in somatic cells were frequently found in urothelial cell carcinomas (urinary tract), and sporadically in adenocarcinomas (colon), squamous cell carcinomas (cervix, nasopharynx, mouth) and multiple myeloma[107, 108]. It was in 1999 that the group of François Radvanyi reported for the first time the presence of *FGFR3* mutations in bladder cancer[109]. All identified mutations were identical to the germline mutations that cause TD I and II (R248C, S249C, Y375C, K652E). TDI and TDII are lethal forms of dwarfism, suggesting that these mutations represent highly activated forms of the receptor. Several studies since have identified 11 different mutations of the FGFR3 gene

Figure 1.7

Achondroplasia caused by activated FGFR3 mutations in germline. These mutations have profound effects on the growth of the long bones. Premature differentiation of chondrocytes is induced in the growth plates causing dwarfism. (This picture is credited to UPI/Bettman, source: website Prof Dr Steven Carr, department of biology, Memorial University of Newfoundland, St. John's Canada; www.mun.ca/biology/scarr/Research)

region. *FGFR3* mutations in bladder cancer are strongly associated with low tumour grade and stage[110-112] and genomically stable tumours[107, 113, 114]. Furthermore frequent mutations have been detected in benign skin lesions (seborrhoeic keratoses and epidermal nevi). These findings in skin lesions, and the preponderance of mutations in PUN-LMP or NMI UC together with the apparent lack of mutations in other malignancies, indicates an association of *FGFR3* mutations with low risk cancers and benign epithelial overgrowths[107, 115-120]. The lack of an association of *FGFR3* mutations in bladder cancer with LOH at 4p16, the location of the FGFR3 gene, corresponds best with its putative role as an oncogene. It is therefore unlikely that *FGFR3* functions as a tumour suppressor gene[117].

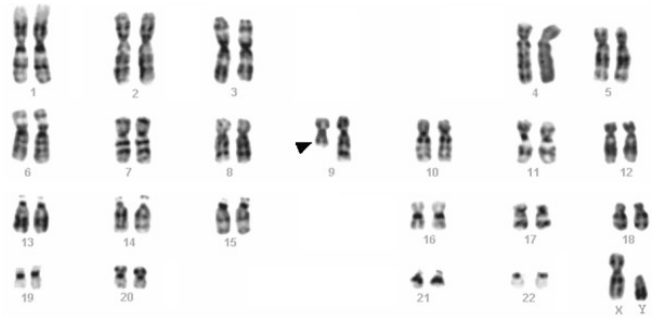
5.3.2 Chromosome 9 and loss of heterozygosity

Loss of heterozygosity (LOH) of chromosome 9 is found in more than 50% of all bladder cancer tumours regardless of grade and stage[121-124] (Figure 1.8a and 1.8b). Because chromosome 9 alterations 1) may be present in morphologically normal urothelium of patients with bladder cancer and 2) may represent the most frequent genetic event in bladder cancer, alterations in chromosome 9 are considered an initiating or otherwise a very early event in early bladder tumourigenesis[125, 126]. Many UC demonstrate LOH of the entire chromosome, suggesting loss of function of tumour suppressor genes on both chromosome arms. Thus, identification of these genes is considered vital to aid understanding of disease pathogenesis and to provide useful clinical markers and targets. Somewhat disturbing, however, to date no definite tumour suppressor gene on chromosome 9 has been identified.

5.4 Theories concerning bladder carcinogenesis

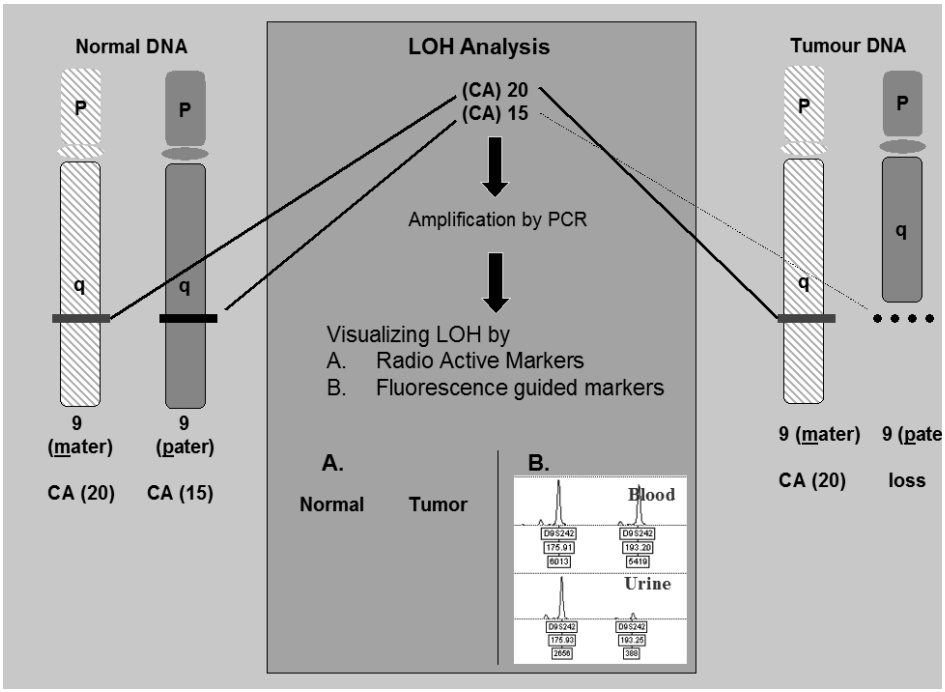
Simultaneous or metachronous development of multifocal tumours with identical or variable histology in the urothelial tract of one patient is a well-known characteristic of UC[47, 49, 53]. Similar observations of multifocal premalignant or malignant lesions have been reported

Figure 1.8a



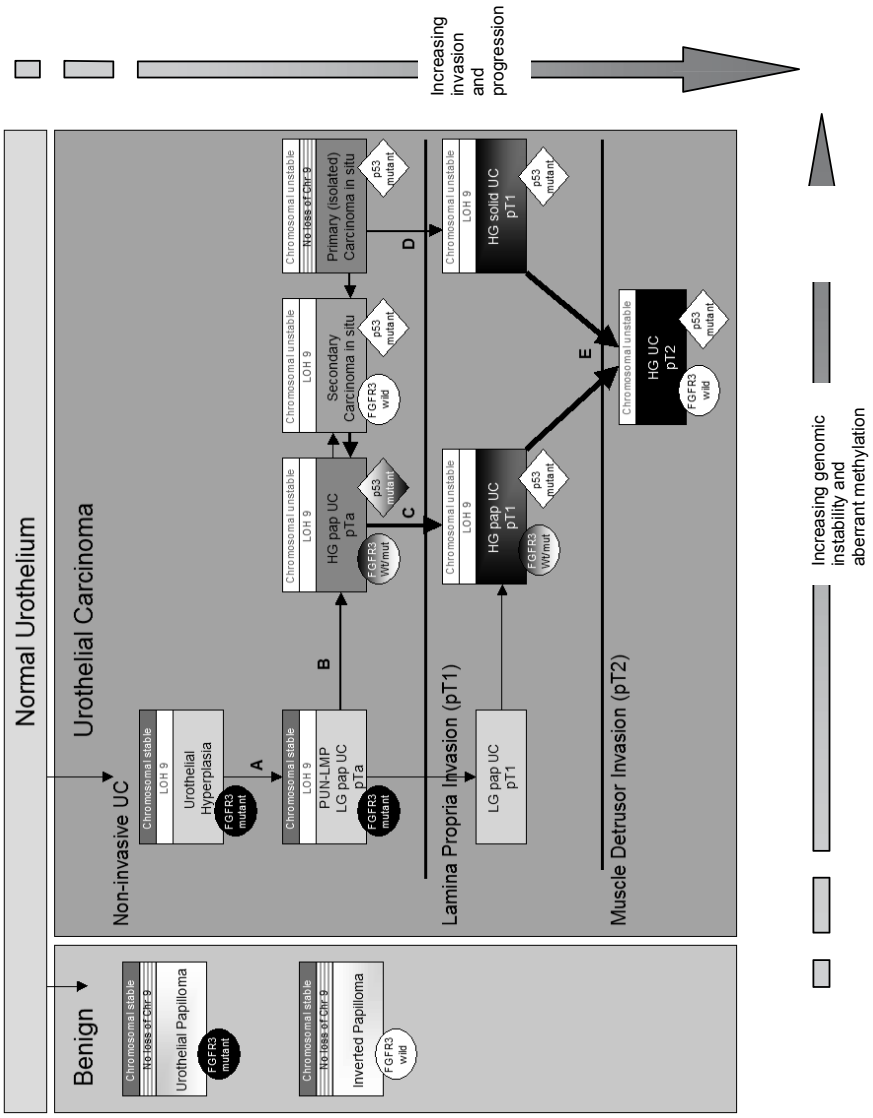
A karyotype of a tumour with a deletion (LOH) of a part of the long arm (9q) of one of the chromosomes 9.

Figure 1.8b



Schematic overview of Loss Of Heterozygosity analysis (LOH); the m(aternal) and p(aternal) copy of chromosome 9 of normal tissue (left) and abnormal tumour tissue (right) are shown. Alterations (i.e. loss of parts) of chromosome 9 are frequent events in bladder cancer. The CA repeats (depicted as dark bars in the p-arm of the chromosomes) often differ in length in the maternal (m, CA(20)) and paternal (p, CA(15)) copy of the chromosome, a phenomenon known as heterozygosity. Loss of heterozygosity (LOH) means that the chromosome lost one of the parts of the short (p-arm) or long (q-arm) of the chromosome containing such a particular CA repeat. The CA repeats of both normal and tumour tissue can be amplified by PCR (Polymerase Chain Reaction), subsequently are the specific CA-repeat parts labeled by radioactive (A) or fluorescent (B) labels. Because the pat and mat fragments containing the CA repeats differ in length, their presence and quantity can be identified in tumour and normal tissue (A) on a gel and quantified by the intensity of the radioactive labels in the gel or (B) by the height of the peaks in the fluorescence detection using a Genetic Analyzer.

Figure 1.9



Hypothetical model for bladder cancer progression showing the molecular pathways of tumorigenesis, partly adapted from Th van der Kwast (Scand J of Urology and Nephrol, 2008) with permission. The thickness of the arrow represents the likeliness of frequency of occurrence. A, B, C, D and E represent morphological transitions characterized by specific genetic and epigenetic alterations. A: activation of *RAS*, *PIK3CA* mutation; B: loss of *RASSF1A,* deletion of the *INK4a* locus; C: loss of *RASSF1A;* D: activation of *RAS,* deletion of the *INK4a* locus, loss of *RB* expression; E: activation of *RAS,* loss of chromosome 8p, expression of extracellular matrix remodelling genes. It is further assumed that *FGFR3* mutation in tumours will provide an advantage in early proliferation, while in tumours with a wild type *FGFR3* gene, activating RAS genes and homozygous deletion of the *INK4a* locus (depending on the region in which p53 is mutated) will cause a growth-inducing signal. FGFR3 (*Fibroblast Growth Factor Receptor 3 gene* mutation; wild type or mutant), UC (Urothelial Carcinoma), PUN-LMP (Papillary Urothelial Neoplasm of Low Malignant Potential), p53 (*p53 gene* muations).

in the oral cavity, respiratory epithelium, and Barrett's esophagus. Macroscopic "normal" urothelium shows in many cases areas of microscopic dysplasia[50].

The frequent multifocality and multiple recurrences of bladder cancer raised the question of the clonal nature of the spatially and temporally distinct UC. Two main theories have been put forward to explain this phenomenon: the field cancerization theory and the monoclonality hypothesis. The "field cancerization" theory defends the hypothesis that the entire urothelium is unstable and many different clones of altered cells are present that give rise to unrelated polyclonal tumours. Many of the observed associations between bladder cancer and various external factors (i.e. tobacco use, aniline dyes) can be subsumed in this genetic theory of carcinogenesis. Carcinogens affect the urothelium at multiple sites, leading to numerous mutations and independent growth of multifocal nonrelated tumours[127].

The other theory describes tumours developing from descendants of a single transformed cell, the monoclonality hypothesis. Most molecular genetic studies confirmed this second hypothesis as they demonstrated monoclonality of multiple tumours within the same patient. The presence of multiple shared genetic changes in all tumours resected from one individual patient suggests that these lesions are related and have most likely evolved from a single altered cell clone. Furthermore, precursor lesions like dysplasia and carcinoma in situ (CIS) generally display the same genetic alterations as the accompanying UC[128-130]. Divergence between the genetic changes found among multiple lesions has been used to determine the timing of the events in tumourigenesis. The assumption is that cancers develop increased numbers of additional genetic changes when they are distantly related as compared to closely related cancers. In this way, a chronological pedigree of multiple recurrences within the same patient could be generated[131]. Strikingly, these pedigrees demonstrate that the earliest clinically manifest bladder cancer in a patient may not necessarily represent the ¨genetically youngest¨ cancer (Figure 1.9).

The group of Hartmann reported that in some patients oligoclonality might explain the presence of multifocal UC in a single patient, an observation in line with the field cancerization hypothesis[132]. However, in a later paper the same group concluded that multiple metachronous tumours shared a monoclonal origin based on their genetic relations[133].

In some cases an upper urinary tract UC may develop *after* the clinical detection of a tumour in the urinary bladder, and this may seem to contradict the hypothesis of intraluminal seeding. It should be noted here, that the time of diagnosis may not necessarily correlate with the time of tumour development, as explained above. Recently, Hoglund combined the field cancerization and monoclonal hypotheses into one model. He hypothesises that the tumour process is initiated by genetically altered but histologically normal cells producing fields of altered cells by intraepithelial displacement. Accumulation of further genetic alterations generates fields of altered urothelium that may reach a state of criticality, and which locally may produce frank tumours[134].

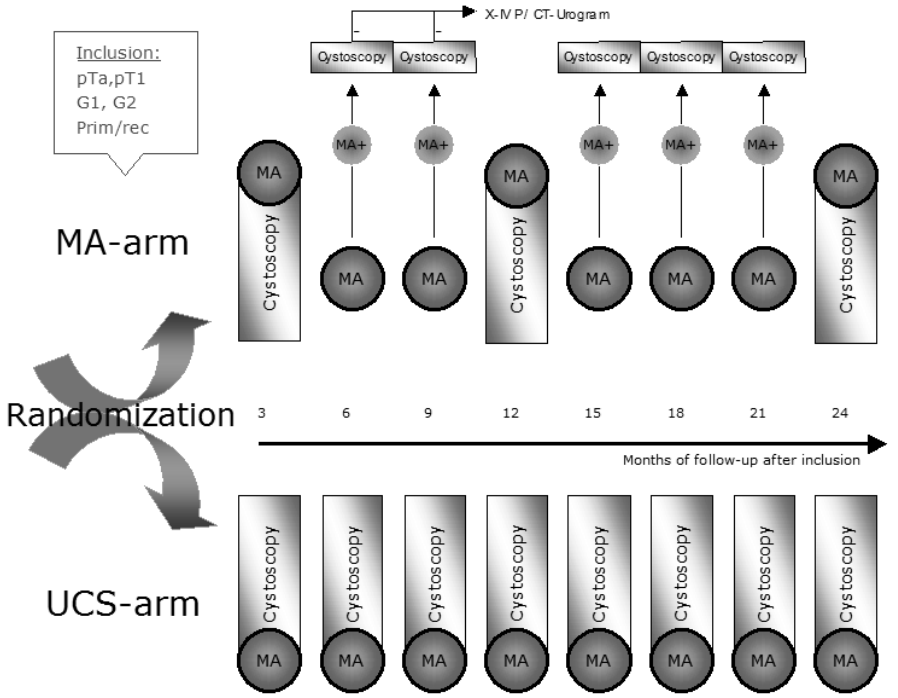
5.4.1 Molecular pathways of bladder carcinogenesis

Non-invasive UC can have two distinct morphologies: the papillary, low-grade, multifocal, highly recurrent, rarely progressive pTa tumours and those with a flat morphology (dysplasia, CIS) frequently progressive to invasive carcinomas. Molecular studies have identified distinct genetic and epigenetic changes in these groups, which led to the identification of at least two major molecular pathways of bladder cancer. *TP53* mutations are often present in CIS lesions and deep muscle invasive (pT2 bladder cancers, but generally not in pTa cancers[135]. *FGFR3* mutations were found to be commonly present in tumours of low stage and grade and were generally absent in tumours with *TP53* mutations, in line with a mutually exclusive two pathway model of carcinogenesis[136]. This concept is confirmed by studies relating histological and molecular characteristics of UC with proliferative activity of the cancers, as measured by the Ki-67 labeling index. Tsuji et al showed that a high Ki-67 labeling index correlates with p53 nuclear accumulation in UC[137]. Van Rhijn et al showed that the favorable *FGFR3* mutation was strongly related to a low Ki-67 proliferation index and he reported that a molecular grading system, based on a combination of *FGFR3* mutation analysis and Ki-67 labeling index was superior to other parameters considering disease free survival[138]. Barbisan et al recently reported that a strong immuno-histochemical expression of FGFR3, a superficial staining pattern of CK20, which is indicative of adequate terminal differentiation by the carcinoma cells, and a low proliferative activity define those papillary urothelial neoplasms of low malignant potential that do not recur[139]. A further description of the Ki-67 labeling index and CK20 staining as biomarkers for bladder cancer will be given in paragraph 7.2.3. Further corroborating the concept of a dual bladder carcinogenesis was the observation by Lindgren et al that *FGFR3* mutant and wild type bladder cancers were highly distinct with regard to their gene expression signatures as assessed by cDNA expression profiling studies[124]. *FGFR3* mutations in NMI UC probably provide a growth advantage in early tumour development. In low-grade papillary tumours without this mutation, activation of other genes, like e.g. the ras oncogene may cause activation of a specific signaling pathway related to growth. Mutant ras proteins can also be trapped in an activated form (just like the gain of function mutant *FGFR3* gene products), which triggers the cell to activate specific signaling pathways resulting in a persistent signal to proliferate. For tumour progression, that is the development of more aggressive tumour behaviour, other genetic events are needed.

Several attempts have been made to apply the molecular and histopathological observations to one comprehensive carcinogenesis model to reflect the possible genetic pathways of UC development. Figure 1.10 shows such a model for bladder carcinogenesis. Most probably this model is not yet complete, but it may provide a useful anchor for further molecular and clinical studies[III].

III Th.H. van der Kwast; How to combine the molecular profile with the clinicopathological profile of urothelial neoplastic lesions, Scandinavian journal of Urology and Nephrology, 2008; 42, 175-184.

Figure 1.10



Follow-up scheme of the randomized clinical trial "Cost-effectiveness of follow-up of patients with non-muscle invasive bladder cancer (CEFUB-trial)."

Our understanding of bladder carcinogenesis still has to deal with a couple of mind-boggling questions. The first concerns the interpretation of CIS. CIS is according to the WHO 2004 a non-invasive intra-urothelial neoplasm, which is considered to represent a premalignant lesion with a very high risk of progression to invasive disease. As a consequence a diagnosis of CIS often leads to an aggressive therapeutic approach by urologists. One study showed a significant difference in genetic alterations between isolated CIS (primary CIS) and secondary CIS, that is CIS associated with a papillary UC. Isolated CIS did not show LOH of chromosome 9, in contrast to most secondary CIS[140]. This may suggest the existence of two distinct forms of CIS associated with different developmental pathways. It is conceivable that CIS found in association with high-grade UC with LOH of chromosome 9 could represent a precursor of the associated papillary tumour. A second important question concerns the enigmatic origin and biological behaviour of pT1 UC. Are these tumours caught in their journey towards the muscle or does this group actually represent the intertwining of the two pathways of bladder carcinogenesis? One author even suggested that pT1 UC may represent a third pathway of

bladder carcinogenesis, since in this subset of cancers *FGFR3* mutations and *TP53* mutations are not mutually exclusive as they may coexist[141]. Indeed, pT1 UC is a clinically very heterogeneous disease, and it is difficult to distinguish the aggressive forms from the less aggressive ones. About 15% of pT1 UC are thought to have metastasized already at the time of their diagnosis. Some clinicians tend to treat pT1 UC by radical treatment early on, and others have a more conservative approach, by lack of robust parameters, which may distinguish low and high-risk pT1 UC.

6. Surveillance in non-muscle invasive bladder cancer

6.1 (Urethro-) Cystoscopic Surveillance

At the time of first diagnosis approximately 70-80% of the patients with bladder cancer presents with non-invasive papillary U (pTa) or with early (non muscle) invasive UC (pT1). As a consequence the fast majority of patients with newly detected UC will undergo local, bladder preserving therapy like transurethral resection (TUR) and intravesical immuno/chemotherapy. The likelihood that these tumours recur is 70% in 5 years with its peak incidence in the first year after initial treatment. Up to about 30% of these recurrent tumours will present with a higher histologic grade and about 5-10% will progress to muscle-infiltrating (pT2) UC[142]. Muscle infiltrating (pT2) UC are life-threatening cancers with a 50% risk of metastatic disease in 5 years. Therefore, patients are recommended to adhere to regular (urethra)-cystoscopic surveillance (UCS) to detect recurrent tumour at early stage. The effectiveness of UCS to improve UC survival has been shown unequivocally[19, 21, 143]. The European Association of Urology (EAU edition 2008) now recommends 2 cystoscopies in the first year after diagnosis of NMI UC followed by annual cystoscopies for up to five years in patients with pTa low-grade NMI UC (*low-risk*). Patients with pT1 UC and/or CIS, or with high-grade pTa tumours (15% of all patients), who are all at *high risk* for recurrence and progression, are recommended to be monitored by cystoscopy every 3 months in the first 2 years, every 4 months in the third year, every sixth months until 5 years of follow-up, and yearly thereafter. In addition, for this subset of patients, a yearly urography of the upper urinary tract (Intravenous Urogram or Computed Tomography) is advised. Patients with an *intermediate risk* have an in-between follow-up scheme, adapted according to personal and subjective factors. These recommendations are based on retrospective studies, while prospective studies are lacking. As a result considerable practice variation is observed[144].

Given the high prevalence of NMI UC this disease is costly to manage, particularly since cystoscopy is a labour intensive method[21, 143, 145, 146]. Besides cystoscopy is an endoscopic (invasive) procedure. Worldwide many research groups continue to search for alternative methods (urine tests) that are at least as accurate and robust as cystoscopy in the detection of UC recurrence. The aim of these research endeavours is to develop a urine test that can

compete with cystoscopy, the current gold standard for the detection of UC recurrences in patients with NMI UC. Such a test needs to be easy to perform, cost-effective and accurate. This thesis will address the important issue of cost-effectiveness of surveillance of patients with NMI UC using the data from a prospective multicenter trial studying the cost-effectiveness of an alternative surveillance strategy.

6.2 Urinary Markers

Since Papanicolaou introduced the cytological investigation of urine sediment it is clear that a voided urine sample represents an easy source of exfoliated tumour cells and tumour related products like proteins, DNA and RNA that may in theory serve as a marker for the presence of UC. Unfortunately, urine cytology is of limited value for the surveillance of low-grade UC, mainly because of its low sensitivity and also because of a considerable operator dependency[147]. Apparently, urine cytology cannot serve as an alternative to cystoscopy for surveillance of low-grade NMI UC. Many studies have focused on evaluating molecular urinary markers and several new urine-based tests for UC have been developed. The United States Food and Drug Administration (FDA) approved ImmunoCyt and FISH (UroVysion, 2001)[147-149]. Van Rhijn et al reported in a review of the literature on the performance of these urinary markers for surveillance of patients with NMI UC[150, 151]. The markers generally have a higher sensitivity but a lower specificity than conventional cytology. Further, it is clear that for high-grade cancers these urinary markers display an increased sensitivity. In the author's view Cytokeratin (CK) based assays (CYFRA21-1 for fragments of cytokeratin 19), the NMP22 assay, immunocytology (ImmunoCyt, LewisX; cytokeratin 20) and the detection of genetic alterations based on the genetic instability of malignancies (Microsatellite Analysis (MA) and Fluorescence in situ Hybridisation (FISH UroVysion)) in voided urine samples are the most promising urine markers for surveillance. The highest median sensitivities were reported for CYFRA21-1 (85%), cytokeratin20 (85%) and Microsatellite Analysis (82%). The highest specificities were reported for cytology (94%), the BTA test (92%) and Microsatellite Analysis (89%)[150, 151]. No prospective (randomized) trials have been reported to date to demonstrate the efficacy of any of these urine tests. The main problem remains to determine what level of sensitivity and specificity of a potential urine marker is required and accepted by urologist and patient. The accuracy needed for a test will principally depend on the purpose of its use; whether it is solely used for screening an asymptomatic population, or for diagnostic reasons in symptomatic patients or whether it is used as an ancillary test for the surveillance of patients with a previous diagnosis of UC.

6.2.1 Statistic analysis of diagnostic tests

Diagnosis is an essential part of clinical practice, and much medical research is carried out to try to improve methods of detection. In the surveillance of bladder cancer the early detection of recurrent tumours is an important item. The paramount question of surveillance is whether

we improve the prognosis of patients by detecting recurrences earlier when compared to the current methods; therefore we want to know how accurate is an urine test in the early detection of bladder cancer recurrences and how does the urine test relate to the so-called 'gold standard' method cystoscopy in diagnosing the true patient status (diseased or healthy; recurrence or no recurrence).

Table 1.4 shows possible approaches to calculate the quality of the test. Sensitivity (a/a+c) and specificity (d/b+d) percentages show the proportions of patients with normal and abnormal test outcome in relationship to presence and absence of disease as measured by a gold standard. Sensitivity is the proportion of patients *with* recurrent disease that were correctly identified by the test. Specificity is the proportion of patients *without* recurrent disease that are correctly identified by the test. At first sight these simple calculations appear to have answered the question, but we have to keep in mind that we related the test outcome only to the gold standard test outcome, which is in this case cystoscopy, the point of reference. In other words we assume that the cystoscopy results reflect the patient's true status of disease. Currently, there are some reasons to doubt the use of cystoscopy as ¨gold standard¨: If one uses photodynamic blue light cystoscopy instead of the standard white light cystoscopy more bladder cancer recurrences are detected[22]. In this thesis we also will provide data to show that cystoscopy may not represent a true gold standard for detection of UC recurrences.

Another approach to determine the accuracy of the test is by its positive and negative predictive value. How accurate is the test in predicting abnormality. A positive predictive value (a/a+b) is the proportion of patients with a *positive* test result who are correctly diagnosed with a recurrent disease and the negative predictive value (d/c+d) is the proportion of patients with a *negative* test result who could not be diagnosed with a recurrent tumour. These two values give a direct assessment of the usefulness of the test in practice. Unfortunately the lower the risk for recurrence, the more certain we can be that a negative test indicates no recurrence, on the other hand the less certain that a positive test result really indicates a tumour.

Table 1.4

	Tumour +	Tumour not proven		
Test +	A Absolute number of true positive (TP)	B Absolute number of False positive (FP)	A+B	(A/A+B) Positive predictive value (PPV)
Test -	C Absolute number of false negative (FN)	D Absolute number of true negative (TN)	C+D	(D/C+D) Negative predictive value (NPV)
	A+C	B+D	N	
	(A/A+C) Sensitivity	(D/B+D) Specificity		

The main difficulty in the analysis of a diagnostic test is the need to decide how accurate the test should be in order to be clinically relevant. In the surveillance of low-grade non-muscle invasive bladder cancer (pTaT1, G1 G2) we are actually screening an ostensibly 'cured' population with a probability of recurrence of 38% (and risk of progression <5%) in the first year and a probability of recurrence of 62% (and risk of progression 10%) within 5 years[152]. Within the 3-monthly surveillance scheme of patients with bladder cancer the risk of finding a recurrence at a follow-up moment is much lower. In the surveillance of patients with NMI UC we particularly want to avoid false negative results (low sensitivity that is missing cancers), but we are willing to accept a moderate number of false positives results. A concern with false positive test outcomes during surveillance of NMI UC patients is the increased burden for the patient as they lead to unnecessary cystoscopies, imaging and bladder biopsies.

The clinical value of the currently available urine tests remains uncertain as data from large prospective multicenter trials considering both the costs and diagnostic accuracy are lacking.

6.2.2 Microsatellite Analysis (MA)

The now most commonly used genetic test on urine, the UroVysion test, is based on the detection of genetically altered cells shed in urine by fluorescence in situ hybridization (FISH), a test approved by the FDA in 2001. This test detects chromosomal abnormalities (i.e. aneuploidy for chromosome 3, 7, 17 and loss of 9p21) in tumour cells exfoliated in the urine of patients with bladder cancer by microscopic visualization of individual cells. Since this FISH test may detect chromosomal alterations in both low-grade and high-grade bladder cancers, this test could in theory be suitable for both the first diagnosis of bladder cancers but also for surveillance strategies (low-risk and high-risk adapted schemes). Nonetheless this test is not implemented in routine clinical practice. A cost-effectiveness study has recently indicated that this FISH test on voided urine samples is not cost-effective[153].

Several authors provided evidence that the use of microsatellite analysis (MA) for detection of cells with loss of heterozygosity (LOH) in voided urine samples may represent a promising tool in diagnosing recurrent UC of the urinary bladder as well as upper urinary tract UC[154-158]. Microsatellites are non-coding, highly polymorphic, short repetitive DNA sequences that are repeated frequently throughout the genome. Because changes in length of microsatellites are frequent in the population it is possible to distinguish between the two copies of a chromosome by using the polymerase chain reaction (PCR) to amplify the repeat region from genomic DNA (i.e. heterozygosity is detected). Loss of one of the chromosomal regions then results in an absent or lower peak of the microsatellite from this chromosome (i.e. loss-of-heterozygosity or LOH). UC is characterised by LOH often displayed at the chromosomal locations: 9q, 9p, 17p (p53 locus), 4p, 8p and 11p. These features of allelic imbalance can be detected by microsatellite analysis (Figure 1.8b). In this technique DNA is extracted from (voided) urine sediments and analysed for the presence of LOH of up to 20 polymorphic

markers from chromosomal regions known to show a high percentage of LOH. Several studies have demonstrated the value of MA in the detection of recurrent UC. A phase II trial performed in our laboratory on patients with NMI UC ('superficial' bladder cancer) reported a sensitivity of MA on voided urine samples of 75% and a specificity of 82%[154-158]. A prospective trial is the obvious next step to demonstrate that MA on voided urine samples of patients with NMI UC is sufficiently accurate and cost-effective for implementation as a routine test. The main focus of this thesis is the evaluation of MA as a surveillance tool in patients with NMI UC and the cost-effectiveness of the test, which we studied in a prospective multicenter trial (CEFUB-trial) conducted from 2002 to 2006.

6.2.3 Outline of a randomized clinical trial

The main idea of a clinical trial is to compare groups of patients who differ only with respect to their surveillance method. Two arms of patients monitored by two different surveillance schemes can be compared; one arm testing a new method, the urinary test, and another control arm where patients are monitored by the 'gold standard,' cystoscopy. In order to avoid bias, both groups may only differ in their surveillance scheme. Patients are randomly assigned to their surveillance group, consequently independent of the characteristics of the tumour and the patient; every patient has the same chance of receiving either surveillance method. The *FGFR3* gene mutation status, histological stage and grade of the tumour are strong prognostic factors in UC, by incorporating these variables in a stratified randomisation scheme these variables can be controlled at the start of the trial.

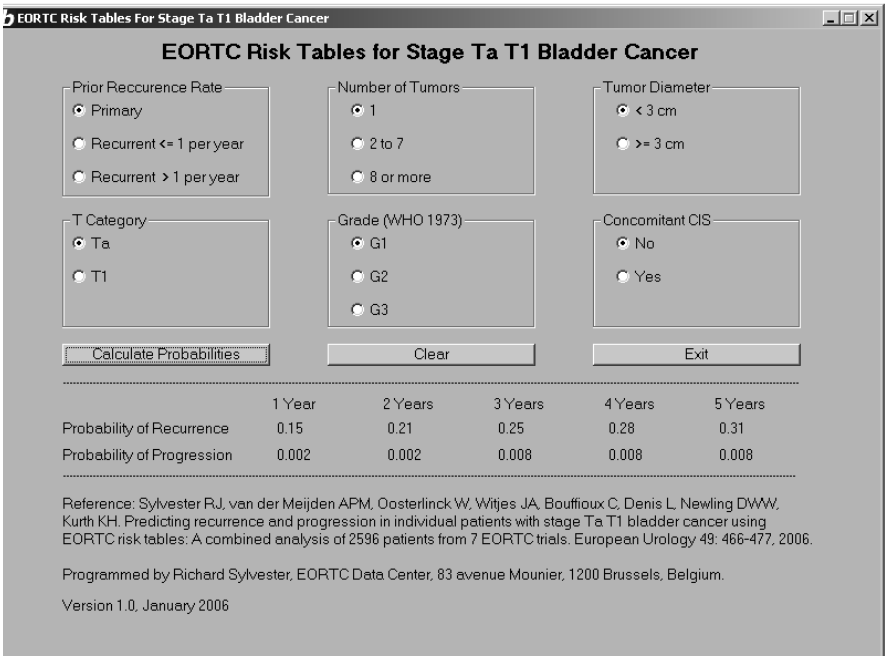
This thesis reports on a randomized clinical trial, in order to evaluate cost-effectiveness of the follow-up of patients with low-grade (WHO 1973 grade 1 or 2) NMI UC by either a conventional regimen (control arm) or a follow-up scheme in which the cystoscopies at 6, 9, 15, 18 and 21 months of follow-up after the last recurrence were replaced by microsatellite analysis (MA) on DNA extracted from voided urine samples (test arm). Primary outcome measure is the number of tumour recurrences in the patients randomized to the test arm and the control arm. In addition, the proportion of patients with tumour progression has been determined. Detection in the test arm of significantly more pT2 tumours would imply that MA detects tumour recurrences at a time that a recurrence had progressed too far. If significantly more low stage tumour recurrences were found in the test arm this might imply that the MA test is more sensitive than cystoscopy. Secondary outcome measures are 1) the quality of life during follow-up of the patients, including physical complaints as a consequence of the diagnostic intervention and 2) comparison of the cost-effectiveness of the two arms of the trial; using the direct and indirect costs involved with the diagnostic interventions and 3) the identification of clinico-pathologic and molecular parameters that may identify low-risk UC patients which may require less frequent follow-up, either by cystoscopy and/or by MA. Patients needed to undersign the informed consent to participate in the trial that they agreed to be randomized.

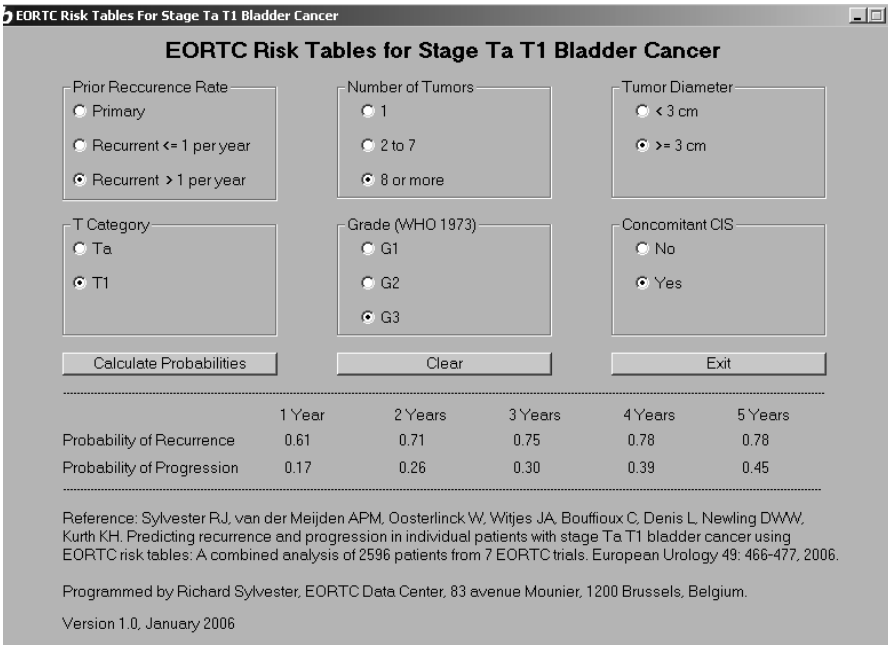
After randomisation patients were stratified for FGFR3 gene status, histological grade and stage of the last tumour and hospital. The flow chart in figure 1.11 provides a scheme depicting the interventions in the control and the test arm of the randomized CEFUB trial.

7. The prediction of the disease course in NMI UC

As mentioned before a more individual-tailored follow-up scheme for patients with NMI UC dependent on their risk profile would be of help to reduce patient burden and costs of surveillance. A more accurate prediction of risk of recurrence and particularly of progression in the individual patient would enable such a person-adjusted follow-up scheme. Lutzeyer and Heney demonstrated in 1982 the clinical value of a number of conventional clinico-pathological prognostic factors, including multifocality, location, site of tumour, pathological grade and stage[159, 160]. Many studies have been conducted since to identify and quantify prognostic factors for the risk of recurrence[35, 46, 56, 142, 160-166], risk of progression[46, 56, 160, 167] and mortality risk[46, 168-170] in patients with NMI bladder cancer[171, 172]. A first step towards individualizing the follow up policy of NMI bladder cancer is the search for prognostic factors to classify patients into different risk groups. Millan-Rodriguez was able to categorize patients into three risk groups, low-risk (50%), intermediate risk (35%) and high risk (15%), by using clinico-pathological prognostic factors[56]. These three risk groups however do not differentiate between risk of recurrence and progression. Oosterlinck et al evaluated the same conventional clinico-pathological factors to classify NMI UC into low, intermediate and high-risk groups for recurrence and progression separately to enable the appropriate choice of adjuvant therapy[173]. However the need to predict more accurately the short-term and long-term risks of both recurrence and progression in individual patients remained. Therefore the European Organization of Research and Treatment of Cancer (EORTC) developed a scoring system using risk tables based on the six most significant clinical and pathological factors; number of tumours, tumour size, prior recurrence rate, stage, presence of CIS and grade[152] (Figure 1.12). Although these tables are currently in clinical use, the search continues for better prognostic factors of progression and particularly of recurrence. Besides, continuous efforts are undertaken to improve the accuracy of prediction of progression and recurrence. Most of these studies focus on the identification of molecular tissue biomarkers as tools to predict the clinical course. Currently proven molecular and genetic predictors for UC recurrence are being weighed for their additional value in risk-profiling (such as *FGFR3* gene mutations, *TP53* gene mutations, deletions of chromosome 9, immuno-histochemical staining of CK20, Ki-67 labeling index, p27-index, etc)[98, 100, 103, 104, 112, 136, 174-180].

Figure 1.11





EORTC risk score table allowing the calculation of the risk of recurrence and progression in individual patients using six conventional clinico-pathologic parameters (reprinted with permission of RJ Sylvester; www.eortc.be)

7.1 What is a clinically relevant independent prognostic factor?

In NMI UC a prognostic factor is defined as a characteristic associated with prognosis and outcome (recurrence and progression). A prognostic factor, a prognosticator, is clinically relevant if it describes the relation with an unknown variable (recurrent disease, progression) independent from other factors (grade, stage, biological markers) and thus is able to improve significantly the prediction of the clinical course of a patient with bladder cancer. Due to the availability of modern high-throughput technology many research groups tend to be more focused on the discovery of candidate markers rather than on their clinical utility. A prognosticator needs to be reproducible, easy to handle (feasible) in clinical practice and applicable in a broad spectrum of clinical settings. As pointed out in a review by Birkhahn et al a combined analysis of a panel of markers spanning different pathways may become the most promising approach to develop a powerful prognostic tool[181].

7.2 Prognostic Factors

7.2.1 Clinical Characteristics

Clinical features as age and comorbidity are not considered as prognosticators for recurrences or progression in NMI UC, in contrary to gender and smoking behaviour, both of which have been studied for their potential impact on the prognosis of NMI UC[6, 8, 9]. Studies have consistently shown that smokers have on an increased risk for bladder cancer compared to non-smokers. Moreover women who smoke comparable amounts of cigarettes may have a higher risk of bladder cancer than man[9]. The proportion of bladder cancer cases attributable to ever smoking was 0.66 (0.61-0.70) for all men and 0.73 (0.66-0.79) for men younger than 60[7].

Irritative bladder symptoms can be indicative for tumours localised in the trigone or CIS or for a large tumour volume reducing the bladder capacity. The latter two are associated with increased risk of progression. Large tumours (>5 cm), multifocality and tumours with diffuse appearance have a higher risk of recurrence[182]. In case of a history of recurrent tumours, the probability of future recurrences increases to approximately 80%. Short disease free interval is also indicative for future recurrences. The risk of recurrence decreases with each normal cystoscopy and is less than 10% at 5 years and extremely low at 10 years if all interval cystoscopies had been normal. Patients with multifocal tumours in the bladder or tumours involving other sites of the urothelial tract (i.e. renal pelvis, ureter, urethra) are at increased risk for recurrence, progression or death due to disease[35, 152, 159, 167].

7.2.2 Pathological Characteristics

Histological grade is a powerful prognosticator for progression in NMI UC, but of limited and disputable value for recurrence[183-185]. PUNLMP has a moderate risk for recurrence (35%) and a very low risk for progression in stage[58, 59, 185, 186]. Patients with PUNLMP have essentially a normal age-related life expectancy. Low-grade NMI UC recur frequently (up to 70%) but they

progress in stage only in up to 12% of the patients[57-59]. The prognosis of high-grade NMI UC is strikingly different. Tumours frequently progress in stage and the mortality due to disease can be as high as 65%[187, 188]. Presence of dysplasia and concomitant CIS are associated with a much-increased risk for progression in stage and death due to disease[60, 189, 190].

Stage is a strong prognosticator for recurrence and progression in NMI UC. The prognosis of patients with pTa tumours is mainly defined by the grade of differentiation. Patients with a single, small, low-grade pTa tumour without CIS are at low risk for both recurrence and progression. Patients with high-grade pTa tumours are at high risk of progression. Most pTa tumours are however well differentiated. Most pT2 and higher staged tumours are high-grade, and the prognostic significance of grade as a risk factor for metastatic disease an disease specific survival is limited. Muscle invasive (pT2) UC have a 50% risk of metastatic disease. In pT1 UC the UC tumour cells invade the lamina propria but they do not invade into the muscularis detrusor. Their biological behaviour is highly variable and unpredictable with the current grading. Stage pT1 tumours frequently are high-grade and they show a recurrence rate of 80%, a progression rate of 60% and a 10-year survival rate of 35%. In efforts to stratify pT1 tumours further, substaging systems have been proposed based on the level of invasion into the lamina propria. Tumours that infiltrate beyond the muscularis mucosae have a higher progression rate[37, 45, 47, 191]. An alternative is to stratify patients according to the level of invasion into the lamina propria measured by a micrometer attached to the microscope[192-194]. A major problem with these substaging systems is their poor reproducibility among pathologists. Further, the lack of orientation of transurethrally resected tissue samples often precludes the possibility to substage according to the level of invasion relative to the vascular plexus an the muscularis mucosae. For this reason we developed in this thesis a more simplified substaging system, which we tested on a series of primary pT1 UC.

Lymphatic and/or vascular invasion is associated with decreased survival in pT1 tumours (44% 5-year survival). However vascular invasion is frequently over-diagnosed[195]. Nevertheless, pathologists report this parameter if identified.

7.2.3 Molecular Characteristics

The encountered limitations of the conventional characteristics have challenged many research groups to discover molecular markers, which can classify bladder cancer in more detail in order help in the selection of the optimal treatment. More specifically, the identification of molecular markers predictive of recurrence and progression is now considered of utmost importance to enable accurate risk stratification for a given patient. Below we describe the scientific and clinical evidence for the potential use of a limited number of biomarkers used in this thesis.

7.2.3.1 FGFR3 gene mutations

One of the most promising prognosticators of NMI UC is the mutation status of the fibroblast growth factor receptor 3 (FGFR3)[99, 100, 103, 104, 107-109, 111, 112, 136, 191]. Ostensibly, mutant *FGFR3*

represents an oncogene, as the mutations activate the FGFR3 signaling pathway. However in UC this mutation is associated with genetically stable bladder cancer and with low-grade, low stage disease[99, 102, 104, 110, 113, 178, 196]. The presence of an *FGFR3* mutation is an indicator for favourable prognosis as the risk to progress in disease is much lower in the presence of this mutation[107, 113, 114]. The potential of *FGFR3* mutation status as a prognosticator for recurrences used to be somewhat controversial, but most studies now agree that the presence of a FGFR3 mutation is not strongly associated with an altered frequency of recurrences. In a large Spanish study a series of 772 UC was analysed for *FGFR3* gene mutation and only the subset of patients with mutant pTaG1 UC showed a slightly higher risk of recurrence[102].

7.2.3.2 TP53 tumour suppressor gene

By definition, a tumour suppressor gene provides a growth advantage to the affected cells in case of its reduced expression or its inactivation. Alterations in the cell cycle regulation pathway are thought to underlie the development of UC. The gene has been mapped to chromosome 17p13 and encodes for a 53kDa protein. This protein plays a protective role in several pivotal cellular processes including regulation of the cell cycle, response to DNA damage, cell death and stimulation of neovascularization[197]. In response to DNA damage, the wild type TP53 stops the cell cycle in order to offer the cell time to repair its DNA or to induce apoptosis in case the repair mechanism fails (cell death). Therefore the gene has been referred to as the 'guardian of the genome'[198]. This protective mechanism does not work anymore if the *TP53* gene is mutated or both alleles are eliminated by deletion (homozygous loss): DNA damage goes unrepaired, mutations become fixed in dividing cells, and the cell turns onto a one-way street that leads to progressive genetic instability.

TP53 alterations are the most common genetic defects found in human cancer. Missense mutations in the *TP53* tumour suppressor gene result in the incorporation of non-cognate amino acids. These changes result in a change in the 3D structure of the TP53 protein. The half-life of the altered protein increases because its degradation is inhibited; this leads to nuclear accumulation of the protein. All human cells express TP53 but, in non-cancerous cells, the short half-life (6-30 minutes) prevents the TP53 protein from accumulating in the nucleus. Often *TP53* mutations can be detected by immunohistochemistry because of this abnormal nuclear accumulation. Numerous studies highlight the frequent nuclear TP53 accumulation in less differentiated and more advanced UC[199, 200]. Lamy et al found inactivating *TP53* mutations in 10% of pTa, 42% of pT1 and 58% of pT2 tumours[141]. Nevertheless, sofar its prognostic impact additional to conventional clinico-pathological markers is limited, and staining for TP53 is not a standard procedure in pathology diagnostics of bladder cancer. The main reason is the highly variable immunostaining outcome, hampering the interpretation of staining results in a given tumour.

7.2.3.3 Ki-67

Proliferative activity has frequently been studied as a potential prognosticator in urothelial carcinomas[201, 202]. The Ki-67 protein is a cellular marker for proliferation, which is strictly associated with cell proliferation. The protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent in resting cells (G0). The monoclonal antibody anti-Ki-67 reacts exclusively with the nuclei of the proliferating cells[203, 204]. Ki-67 is an excellent marker to determine the growth fraction of a given cell population. The fraction of Ki-67-positive tumour cells (the *Ki-67 labelling index*) has been shown to be associated with the clinical course of several cancers, including bladder cancer.

One of the monoclonal antibodies most commonly used in clinical applications to determine the *Ki-67 labelling index* is MIB-1[205]. Increased proliferative activity reflected by MIB-1 immuno-histochemical staining increases with tumour stage and grade and is a prognostic factor in disease progression and recurrence[206]. Tsuji et al showed that high Ki-67 index correlates with p53 nuclear accumulation in urothelial bladder carcinoma[137]. Asakura et al found Ki-67 as a prognostic factor for recurrence and progression in non-muscle invasive bladder carcinoma[207]. In pTa/T1 tumours, Ki-67 labeling index and expression of cyclins D1 and D3, which are upstream regulators of the cell cycle, might be relevant predictors of survival[208]. Nevertheless, in pTa G2 tumours, Holmang et al did not observe any correlation between high progression rate and high labeling index[58]. In spite of these contradictory results this biological marker continues to be a potential prognosticator that might be implemented in the pathological routine of bladder cancer diagnostics. Importantly, Van Rhijn et al of our laboratory successfully incorporated the Ki-67 labeling index in their molecular grading system of bladder cancers[98].

7.2.3.4 CK20 protein expression

Cytokeratins are intermediate filament proteins present in normal epithelia and their expression is maintained during malignant transformation. CK-antibodies are exploit to identify the primary site of epithelial tumours. CK20 is one of 20 cytokeratin subtypes that is expressed in mature enterocytes and goblet cells of intestinal mucosa, and Merkel cells of the skin. In addition, specifically umbrella cells of the normal urothelial lining, but not basal or intermediate urothelial cells express CK20. Therefore CK20 expression is related to a normal differentiation and maturation. In UC CK20 expression may be aberrant, which becomes manifest by its complete absence of staining or by involvement of multiple cell layers with > 10% of the cells stained. When CK20 expression in non invasive UC is limited to the most superficial (umbrella) cells, a normal expression pattern, it is associated with a mild disease course, whereas expression in >10% of the cells of the entire urothelial thickness is associated with higher tumour grade and an increased risk of recurrence and progression[100, 209-211]. Its independent prognostic role continues to be a controversial issue.

8. Patients' Opinion

Quality of life

Quality of life is the degree of well-being felt by an individual or a group of people (Wikipedia). Quality of life is not a concrete topic in science, and cannot be measured directly. Two components of quality of life must be considered; the physical component (pain, morbidity, possibilities to protect against disease etc) and the emotional component (stress, fear,pleasure etc). Although it is difficult to quantify quality of life issues, one can assume that the higher average level of physical and emotional experiences a person has, the better overall quality of life is experienced.

Today quality of life issues play a pivotal role in todays health care. A growing field of research is dedicated to the development, evaluation and application of quality of life measures within health related research (e.g. within randomized controlled trials). Many of these research efforts focus on the quantitative measurement of health related quality of life (HRQoL). They also focus on measuring HRQoL from the perspective of the patient and thus take the form of self completed questionnaires.

The role of HRQoL is increasing. Patient satisfaction and preferences together with utility assessment are particularly important when it comes to the selection of new diagnostic and/or therapeutic regimens. Not much is known regarding the burden imposed by bladder cancer upon patient HRQoL. We all assume that the HRQoL related to the diagnosis of bladder cancer depends a) on the extent (stage) of the carcinoma and corresponding therapies and b) on the intensity of the surveillance and the surveillance method in case of NMI UC. Several studies have quantified the differences in quality of life in patients with the diagnosis MI UC regarding bladder sparing surgery, cystectomy and urinary diversions[212-216], but only few studies have focused on the influence of the bladder cancer surveillance on the well-being of patients. In this thesis we made an attempt to fill this gap.

Patients diagnosed with NMI UC are recommended to adhere to an intensive surveillance scheme by cystoscopic examinations in order to detect UC recurrences. Cystoscopy is an invasive method which is assumed to be experienced as painfull, burdensome and to have a major impact on patients' general functioning. The introduction of the flexible scope improved patients' perspective on the endoscopic experience[217, 218], but the experience obviously continues to be of invasive nature and potentially distressful for the patient. A few studies investigated the opinion of patients regarding flexible cystoscopic examination and confirmed the assumption that it is painful; when the tip passess the external sphincter[219], irrespective of the analgesic method[220], irrespective of delayed or immediate cystoscopic examination after lidocaine injection[221]. We assume that patients consider an invasive method as less attractive compared to a less invasive method like urinary tests. Further, Stav et al reported a transient impairment of sexual performance until two weeks after cystoscopy[222]. Vriesema et all investigated patients' opinion about the required validity of non-invasive diagnostic tools and concluded that 89% of the patients prefer flexible cystoscopy if sensitivity of a urinary

test is lower than 90%[223]. Yossepowitch et al asked seven years later a similar question and concluded that from a patient perspective a urinary biomarker would likely require a 95% or greater diagnostic accuracy[224]. Hence; what we know so far is that, although patients experience a cystoscopy as painful (of course) they prefer a solid, well-established and accurate method above a non-invasive method in an experiment of thoughts.

Although we still assume that the burden and the impact on general functioning of a cystoscopic examination is so large that we need to offer patients a non-invasive test, we still do not know if the impact on quality of life in bladder cancer patients is due to the diagnosis and the fear of recurrence or due to the prospect of an intensive surveillance scheme. This thesis tries to evaluate to what extent the burden of cystoscopy is outweighed by its better diagnostic properties as compared to non-invasive urine tests.

9. Health Care System

Botteman et al. calculated (based on values in the year 2001) that bladder cancer was the fifth most expensive cancer in overall costs, but the most expensive cancer per patient[19, 143], caused by the long-term survival and life-long monitoring and treatment of individual patients. They concluded that current diagnostic processes, follow-up schedules and treatment are very expensive, especially for NMI UC, which can be considered a chronic disease. Avritscher et al confirmed these findings, and thereby demonstrated in a single-institution cohort that approximately 60% of the costs were related to surveillance and treatment of recurrences and 30% to complications. Transurethral resections of (recurrent) bladder cancers proved to be the strongest contributor to the costs of surveillance of patients with a bladder cancer. For this reason, alternatives are being explored, such as in office fulguration of small cancers and expectant management of low-grade low stage cancers. The lifetime cost of bladder cancer was lower for a worst-case scenario (99,270 dollars) than for a best-case scenario (120,684 dollars)[225, 226]. It can be hypothesized that a lowering of the frequency of recurrences, the reduction of the incidence of progression and the disappearance of the number of complications will decrease the lifetime costs of bladder cancer. Further, it may be considered to lower the costs of surveillance by introducing cheaper methods and/or an alternative scheme of surveillance, based, e.g. on an improved patient stratification. In this thesis we will address the question, how much an alternative method or surveillance scheme may cost in order to replace cystoscopy in a cost-effective way.

10. Abbreviations

ALA	Aminolaevulinic Acid
BCG	Bacillus Calmette-Guerin
BTA	Bladder Tumour Antigen
CEFUB-trial	Trial to evaluate the Cost-Effectiveness of Follow-up of Patients with Bladder cancer
CGH	Comparitive Genomic Hybridization
CI	Confidence Interval
CIN	Chromosomal Instability
CIS	Carcinoma in Situ
CK20	Cytokeratin 20
CRF	Case Report Form
CT	Computed Tomography
CYFRA21-1	Fragment of Cytokeratin 21
DNA	Deoxyribonucleic acid
EAU	European Association of Urology
EORTC	European Organization of Research and Treatment of Cancer
FDA	Food and Drug Administration
FGFR3	Fibroblast Growth Factor Receptor 3
FISH	Fluorescence in situ Hybridisation
FN	False Negative
FP	False Positive
HAL	Hexi-aminolaevulinic Acid
HE	haematoxyline-eosine
hpf	high power field
HR	Hazard Rate
HRQoL	Health Related Quality of Life
IVU	Intravenous Pyelogram
LOH	Loss of Heterozygosity
MA	Microsatellite Analysis
MI	Muscle Invasive
MM	Multipele Myeloma
MRI	Magnetic Resonance Imaging
MSI	Microsatellite Instability
NMI UC	Non-muscle invasive Urothelial Carcinoma
NMP 22	Nuclear Matrix Protein 22
NPV	Negative Predictive Value
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PDD	Photo Dynamic Diagnosis
PET	Positron Emission Tomography
PPV	Positive Predictive Value
PUN-LMP	Papillary Urothelial Neoplasm of Low Malignant Potential
RR	Relative Risk
SADDAN	Severe Achondroplasia with Developmental Delay and Acanthosis Nigricans
TD	Thanatophoric Dysplasia
TNM	Tumour-Node-Metastases at distance
TUR	Trans Urethral Resection
UC	Urothelial Carcinoma
UCS	Urethro-Cystoscopic Surveillance
UICC	Union International Contre le Cancer
UUT	Upper Urinary Tract
VAS	Visual Analog Scale
WHO	World Health Organisation

11. References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
2. Sengupta N, Siddiqui E, Mumtaz FH. Cancers of the bladder. J R Soc Health 2004;124:228-9.
3. Pashos CL, Botteman MF, Laskin BL, Redaelli A. Bladder cancer: epidemiology, diagnosis, and management. Cancer Pract 2002;10:311-22.
4. Oosterhuis JW, Schapers RF, Janssen-Heijnen ML, Pauwels RP, Newling DW, Ten Kate F. Histological grading of papillary urothelial carcinoma of the bladder: prognostic value of the 1998 WHO/ISUP classification system and comparison with conventional grading systems. J Clin Pathol 2002;55: 900-5.
5. Mungan NA, Kiemeney LA, Van Dijck JA, Van Der Poel HG, Witjes JA. Gender differences in stage distribution of bladder cancer. Urology 2000;55:368-71.
6. Wallerand H, Bakkar AA, De Medina SG, Pairon JC, Yang YC, Vordos D, et al. Mutations in TP53, but not FGFR3, in urothelial cell carcinoma of the bladder are influenced by smoking: contribution of exogenous versus endogenous carcinogens. Carcinogenesis 2005;26:177-84.
7. Brennan P, Bogillot O, Cordier S, Greiser E, Schill W, Vineis P, et al. Cigarette smoking and bladder cancer in men: a pooled analysis of 11 case-control studies. Int J Cancer 2000;86:289-94.
8. Negri E, La Vecchia C. Epidemiology and prevention of bladder cancer. Eur J Cancer Prev 2001;10: 7-14.
9. Castelao JE, Yuan JM, Skipper PL, Tannenbaum SR, Gago-Dominguez M, Crowder JS, et al. Gender- and smoking-related bladder cancer risk. J Natl Cancer Inst 2001;93:538-45.
10. Cheever AW. Schistosomiasis and neoplasia. J Natl Cancer Inst 1978;61:13-8.
11. Mostafa MH, Helmi S, Badawi AF, Tricker AR, Spiegelhalder B, Preussmann R. Nitrate, nitrite and volatile N-nitroso compounds in the urine of Schistosoma haematobium and Schistosoma mansoni infected patients. Carcinogenesis 1994;15:619-25.
12. Lynch CF, Cohen MB. Urinary system. Cancer 1995;75:316-29.
13. Munoz JJ, Ellison LM. Upper tract urothelial neoplasms: incidence and survival during the last 2 decades. J Urol 2000;164:1523-5.
14. Mazeman E. Tumours of the upper urinary tract calyces, renal pelvis and ureter. Eur Urol 1976;2: 120-6.
15. Devesa SS, Silverman DT, Mclaughlin JK, Brown CC, Connelly RR, Fraumeni JF, Jr. Comparison of the descriptive epidemiology of urinary tract cancers. Cancer Causes Control 1990;1:133-41.
16. Nocks BN, Heney NM, Daly JJ, Perrone TA, Griffin PP, Prout GR, Jr. Transitional cell carcinoma of renal pelvis. Urology 1982;19:472-7.
17. Batata MA, Whitmore WF, Hilaris BS, Tokita N, Grabstald H. Primary carcinoma of the ureter: a prognostic study. Cancer 1975;35:1626-32.
18. Herr HW, Donat SM, Dalbagni G. Correlation of cystoscopy with histology of recurrent papillary tumors of the bladder. J Urol 2002;168:978-80.
19. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. Pharmacoeconomics 2003;21:1315-30.
20. Mariappan P, Smith G. A surveillance schedule for G1Ta bladder cancer allowing efficient use of check cystoscopy and safe discharge at 5 years based on a 25-year prospective database. J Urol 2005;173:1108-11.
21. Wright MP, Jones DJ. Surveillance for bladder cancer: the management of 4. 8 million people. South-west Urologists. BJU Int 2000;85:431-3.
22. Witjes JA, Douglass J. The role of hexaminolevulinate fluorescence cystoscopy in bladder cancer. Nat Clin Pract Urol 2007;4:542-9.
23. Filbeck T, Pichlmeier U, Knuechel R, Wieland WF, Roessler W. Do patients profit from 5-aminolevulinic acid-induced fluorescence diagnosis in transurethral resection of bladder carcinoma? Urology 2002;60:1025-8.
24. Filbeck T, Pichlmeier U, Knuechel R, Wieland WF, Roessler W. Clinically relevant improvement of recurrence-free survival with 5-aminolevulinic acid induced fluorescence diagnosis in patients with superficial bladder tumors. J Urol 2002;168:67-71.

25. Filbeck T, Roessler W, Knuechel R, Straub M, Kiel HJ, Wieland WF. Clinical results of the transurethreal resection and evaluation of superficial bladder carcinomas by means of fluorescence diagnosis after intravesical instillation of 5-aminolevulinic acid. J Endourol 1999;13:117-21.
26. Kamat AM, Dinney CP, Gee JR, Grossman HB, Siefker-Radtke AO, Tamboli P, et al. Micropapillary bladder cancer: a review of the University of Texas M. D. Anderson Cancer Center experience with 100 consecutive patients. Cancer 2007;110:62-7.
27. Grossman HB, Gomella L, Fradet Y, Morales A, Presti J, Ritenour C, et al. A phase III, multicenter comparison of hexaminolevulinate fluorescence cystoscopy and white light cystoscopy for the detection of superficial papillary lesions in patients with bladder cancer. J Urol 2007;178:62-7.
28. Fradet Y, Grossman HB, Gomella L, Lerner S, Cookson M, Albala D, et al. A comparison of hexaminolevulinate fluorescence cystoscopy and white light cystoscopy for the detection of carcinoma in situ in patients with bladder cancer: a phase III, multicenter study. J Urol 2007;178:68-73; discussion 73.
29. Kriegmair M, Baumgartner R, Knuchel R, Stepp H, Hofstadter F, Hofstetter A. Detection of early bladder cancer by 5-aminolevulinic acid induced porphyrin fluorescence. J Urol 1996;155:105-9; discussion 09-10.
30. Kriegmair M, Baumgartner R, Knuechel R, Steinbach P, Ehsan A, Lumper W, et al. Fluorescence photodetection of neoplastic urothelial lesions following intravesical instillation of 5-aminolevulinic acid. Urology 1994;44:836-41.
31. Cina SJ, Epstein JI, Endrizzi JM, Harmon WJ, Seay TM, Schoenberg MP. Correlation of cystoscopic impression with histologic diagnosis of biopsy specimens of the bladder. Hum Pathol 2001;32:630-7.
32. Satoh E, Miyao N, Tachiki H, Fujisawa Y. Prediction of muscle invasion of bladder cancer by cystoscopy. Eur Urol 2002;41:178-81.
33. Dutta SC, Smith JA, Jr., Shappell SB, Coffey CS, Chang SS, Cookson MS. Clinical under staging of high risk nonmuscle invasive urothelial carcinoma treated with radical cystectomy. J Urol 2001;166:490-3.
34. Amin MB, Gomez JA, Young RH. Urothelial transitional cell carcinoma with endophytic growth patterns: a discussion of patterns of invasion and problems associated with assessment of invasion in 18 cases. Am J Surg Pathol 1997;21:1057-68.
35. Parmar MK, Freedman LS, Hargreave TB, Tolley DA. Prognostic factors for recurrence and followup policies in the treatment of superficial bladder cancer: report from the British Medical Research Council Subgroup on Superficial Bladder Cancer (Urological Cancer Working Party). J Urol 1989;142:284-8.
36. Amling CL, Thrasher JB, Frazier HA, Dodge RK, Robertson JE, Paulson DF. Radical cystectomy for stages Ta, Tis and T1 transitional cell carcinoma of the bladder. J Urol 1994;151:31-5; discussion 35-6.
37. Zhang GK, Uke ET, Sharer WC, Borkon WD, Bernstein SM. Reassessment of conservative management for stage T1N0M0 transitional cell carcinoma of the bladder. J Urol 1996;155:1907-9.
38. Heicappell R, Muller-Mattheis V, Reinhardt M, Vosberg H, Gerharz CD, Muller-Gartner H, et al. Staging of pelvic lymph nodes in neoplasms of the bladder and prostate by positron emission tomography with 2-[(18)F]-2-deoxy-D-glucose. Eur Urol 1999;36:582-7.
39. Beyersdorff D, Taupitz M, Giessing M, Turk I, Schnorr D, Loening S, et al.[The staging of bladder tumors in MRT: the value of the intravesical application of an iron oxide-containing contrast medium in combination with high-resolution T2-weighted imaging]. Rofo 2000;172:504-8.
40. Caterino M, Giunta S, Finocchi V, Giglio L, Mainiero G, Carpanese L, et al. Primary cancer of the urinary bladder: CT evaluation of the T parameter with different techniques. Abdom Imaging 2001;26:433-8.
41. Vock P, Haertel M, Fuchs WA, Karrer P, Bishop MC, Zingg EJ. Computed tomography in staging of carcinoma of the urinary bladder. Br J Urol 1982;54:158-63.
42. Voges GE, Tauschke E, Stockle M, Alken P, Hohenfellner R. Computerized tomography: an unreliable method for accurate staging of bladder tumors in patients who are candidates for radical cystectomy. J Urol 1989;142:972-4.
43. Buszello H, Muller-Mattheis V, Ackermann R.[Value of computerized tomography in detection of lymph node metastases in bladder cancer]. Urologe A 1994;33:243-6.

44. Bhutani MS, Suryaprasad S, Moezzi J, Seabrook D. Improved technique for performing endoscopic ultrasound guided fine needle aspiration of lymph nodes. Endoscopy 1999;31:550-3.
45. Sylvester RJ. The use of intravesical chemotherapy and possibilities for improving its efficacy. Eur Urol 2006;50:233.
46. Kurth KH, Denis L, Bouffioux C, Sylvester R, Debruyne FM, Pavone-Macaluso M, et al. Factors affecting recurrence and progression in superficial bladder tumours. Eur J Cancer 1995;31A:1840-6.
47. Koss LG. Mapping of the urinary bladder: its impact on the concepts of bladder cancer. Hum Pathol 1979;10:533-48.
48. Farrow GM, Utz DC, Rife CC. Morphological and clinical observations of patients with early bladder cancer treated with total cystectomy. Cancer Res 1976;36:2495-501.
49. Koss LG, Tiamson EM, Robbins MA. Mapping cancerous and precancerous bladder changes. A study of the urothelium in ten surgically removed bladders. Jama 1974;227:281-6.
50. Schade RO, Swinney J. Pre-cancerous changes in bladder epithelium. Lancet 1968;2:943-6.
51. Murphy WM, Soloway MS. Urothelial dysplasia. J Urol 1982;127:849-54.
52. Younes M, Sussman J, True LD. The usefulness of the level of the muscularis mucosae in the staging of invasive transitional cell carcinoma of the urinary bladder. Cancer 1990;66:543-8.
53. Epstein JI, Amin MB, Reuter VR, Mostofi FK. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. Am J Surg Pathol 1998;22:1435-48.
54. Jimenez RE, Keane TE, Hardy HT, Amin MB. pT1 urothelial carcinoma of the bladder: criteria for diagnosis, pitfalls, and clinical implications. Adv Anat Pathol 2000;7:13-25.
55. Paner GP, Ro JY, Wojcik EM, Venkataraman G, Datta MW, Amin MB. Further characterization of the muscle layers and lamina propria of the urinary bladder by systematic histologic mapping: implications for pathologic staging of invasive urothelial carcinoma. Am J Surg Pathol 2007;31: 1420-9.
56. Millan-Rodriguez F, Chechile-Toniolo G, Salvador-Bayarri J, Palou J, Vicente-Rodriguez J. Multivariate analysis of the prognostic factors of primary superficial bladder cancer. J Urol 2000;163:73-8.
57. Alsheikh A, Mohamedali Z, Jones E, Masterson J, Gilks CB. Comparison of the WHO/ISUP classification and cytokeratin 20 expression in predicting the behavior of low-grade papillary urothelial tumors. World/Health Organization/Internattional Society of Urologic Pathology. Mod Pathol 2001;14:267-72.
58. Holmang S, Andius P, Hedelin H, Wester K, Busch C, Johansson SL. Stage progression in Ta papillary urothelial tumors: relationship to grade, immunohistochemical expression of tumor markers, mitotic frequency and DNA ploidy. J Urol 2001;165:1124-8; discussion 28-30.
59. Holmang S, Johansson SL. Stage Ta-T1 bladder cancer: the relationship between findings at first followup cystoscopy and subsequent recurrence and progression. J Urol 2002;167:1634-7.
60. Orozco RE, Martin AA, Murphy WM. Carcinoma in situ of the urinary bladder. Clues to host involvement in human carcinogenesis. Cancer 1994;74:115-22.
61. Prout GR, Jr., Griffin PP, Daly JJ, Heney NM. Carcinoma in situ of the urinary bladder with and without associated vesical neoplasms. Cancer 1983;52:524-32.
62. Rosenkilde Olsen P, Wolf H, Schroeder T, Fischer A, Hojgaard K. Urothelial atypia and survival rate of 500 unselected patients with primary transitional-cell tumour of the urinary bladder. Scand J Urol Nephrol 1988;22:257-63.
63. Weinstein RS, Miller AW, 3rd, Pauli BU. Carcinoma in situ: comments on the pathobiology of a paradox. Urol Clin North Am 1980;7:523-31.
64. Sylvester RJ, Oosterlinck W, Van Der Meijden AP. A single immediate postoperative instillation of chemotherapy decreases the risk of recurrence in patients with stage Ta T1 bladder cancer: a meta-analysis of published results of randomized clinical trials. J Urol 2004;171:2186-90, quiz 435.
65. Bevers RF, Kurth KH, Schamhart DH. Role of urothelial cells in BCG immunotherapy for superficial bladder cancer. Br J Cancer 2004;91:607-12.
66. Bevers RF, De Boer EC, Kurth KH, Schamhart DH. BCG-induced interleukin-6 upregulation and BCG internalization in well and poorly differentiated human bladder cancer cell lines. Eur Cytokine Netw 1998;9:181-6.

67. Dhar NB, Klein EA, Reuther AM, Thalmann GN, Madersbacher S, Studer UE. Outcome after radical cystectomy with limited or extended pelvic lymph node dissection. J Urol 2008;179:873-8; discussion 78.
68. Steven K, Poulsen AL. Radical cystectomy and extended pelvic lymphadenectomy: survival of patients with lymph node metastasis above the bifurcation of the common iliac vessels treated with surgery only. J Urol 2007;178:1218-23; discussion 23-4.
69. Poulsen AL, Horn T, Steven K. Radical cystectomy: extending the limits of pelvic lymph node dissection improves survival for patients with bladder cancer confined to the bladder wall. J Urol 1998;160:2015-9; discussion 20.
70. Vieweg J, Gschwend JE, Herr HW, Fair WR. The impact of primary stage on survival in patients with lymph node positive bladder cancer. J Urol 1999;161:72-6.
71. Sanderson KM, Skinner D, Stein JP. The prognostic and staging value of lymph node dissection in the treatment of invasive bladder cancer. Nat Clin Pract Urol 2006;3:485-94.
72. Minervini R, Morelli G, Fontana N, Minervini A, Fiorentini L. Functional evaluation of different ileal neobladders and ureteral reimplantation techniques. Eur Urol 1998;34:198-202.
73. Osman Y, Abol-Enein H, Nabeeh A, Gaballah M, Bazeed M. Long-term results of a prospective randomized study comparing two different antireflux techniques in orthotopic bladder substitution. Eur Urol 2004;45:82-6.
74. Slojewski M.[Results of radical cystectomy for management of invasive bladder cancer with special reference to prognostic factors and quality of life depending on the type of urinary diversion]. Ann Acad Med Stetin 2000;46:217-29.
75. Huncharek M, Muscat J, Geschwind JF. Planned preoperative radiation therapy in muscle invasive bladder cancer; results of a meta-analysis. Anticancer Res 1998;18:1931-4.
76. Housset M, Maulard C, Chretien Y, Dufour B, Delanian S, Huart J, et al. Combined radiation and chemotherapy for invasive transitional-cell carcinoma of the bladder: a prospective study. J Clin Oncol 1993;11:2150-7.
77. Nowell PC. The clonal evolution of tumor cell populations. Science 1976;194:23-8.
78. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57-70.
79. Renan MJ. How many mutations are required for tumorigenesis? Implications from human cancer data. Mol Carcinog 1993;7:139-46.
80. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. Nature 1998;396: 643-9.
81. Koppert LB, Wijnhoven BP, Van Dekken H, Tilanus HW, Dinjens WN. The molecular biology of esophageal adenocarcinoma. J Surg Oncol 2005;92:169-90.
82. Fodde R, Smits R, Clevers H. APC, signal transduction and genetic instability in colorectal cancer. Nat Rev Cancer 2001;1:55-67.
83. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008;321:1801-6.
84. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, et al. An integrated genomic analysis of human glioblastoma multiforme. Science 2008;321:1807-12.
85. Catto JW, Meuth M, Hamdy FC. Genetic instability and transitional cell carcinoma of the bladder. BJU Int 2004;93:19-24.
86. Mongiat-Artus P, Miquel C, Van Der Aa M, Buhard O, Hamelin R, Soliman H, et al. Microsatellite instability and mutation analysis of candidate genes in urothelial cell carcinomas of upper urinary tract. Oncogene 2006;25:2113-8.
87. Mongiat-Artus P, Miquel C, Van Der Aa M, Buhard O, Hamelin R, Soliman H, et al.[Analysis of the microsatellite instability gene mutation in the urothelial tumor of the upper urinary tract]. Prog Urol 2005;15:1286-92.
88. Mongiat-Artus P, Miquel C, Van Der Aa M, Buhard O, Hamelin R, Bangma C, et al. Infrequent microsatellite instability in urothelial cell carcinoma of the bladder in young patients. Eur Urol 2006; 49:685-90.
89. Mongiat-Artus P, Miquel C, Flejou JF, Coulet F, Verine J, Buhard O, et al. Spectrum of molecular alterations in colorectal, upper urinary tract, endocervical, and renal carcinomas arising in a patient with hereditary non-polyposis colorectal cancer. Virchows Arch 2006;449:238-43.

90. Roupret M, Yates DR, Comperat E, Cussenot O. Upper urinary tract urothelial cell carcinomas and other urological malignancies involved in the hereditary nonpolyposis colorectal cancer (lynch syndrome) tumor spectrum. Eur Urol 2008;54:1226-36.
91. Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer 1993;71:677-85.
92. Kiemeney LA, Kuiper RP, Pfundt R, Van Reijmersdal S, Schoenberg MP, Aben KK, et al. No evidence for large-scale germline genomic aberrations in hereditary bladder cancer patients with high-resolution array-based comparative genomic hybridization. Cancer Epidemiol Biomarkers Prev 2006;15:180-3.
93. Zlowocka E, Cybulski C, Gorski B, Debniak T, Slojewski M, Wokolorczyk D, et al. Germline mutations in the CHEK2 kinase gene are associated with an increased risk of bladder cancer. Int J Cancer 2008;122:583-6.
94. Knowles MA. The genetics of transitional cell carcinoma: progress and potential clinical application. BJU Int 1999;84:412-27.
95. Cordon-Cardo C, Cote RJ, Sauter G. Genetic and molecular markers of urothelial premalignancy and malignancy. Scand J Urol Nephrol Suppl 2000;82-93.
96. Knowles MA. Molecular subtypes of bladder cancer: Jekyll and Hyde or chalk and cheese? Carcinogenesis 2006;27:361-73.
97. Knowles MA. Molecular pathogenesis of bladder cancer. Int J Clin Oncol 2008;13:287-97.
98. Van Rhijn BW, Vis AN, Van Der Kwast Th, Kirkels WJ, Radvanyi F, Ooms EC, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. J Clin Oncol 2003;21:1912-21.
99. Van Oers JM, Zwarthoff EC, Rehman I, Azzouzi AR, Cussenot O, Meuth M, et al. FGFR3 Mutations Indicate Better Survival in Invasive Upper Urinary Tract and Bladder Tumours. Eur Urol 2008;
100. Van Oers JM, Wild PJ, Burger M, Denzinger S, Stoehr R, Rosskopf E, et al. FGFR3 mutations and a normal CK20 staining pattern define low-grade noninvasive urothelial bladder tumours. Eur Urol 2007;52:760-8.
101. Van Oers JM, Lurkin I, Van Exsel AJ, Nijsen Y, Van Rhijn BW, Van Der Aa MN, et al. A simple and fast method for the simultaneous detection of nine fibroblast growth factor receptor 3 mutations in bladder cancer and voided urine. Clin Cancer Res 2005;11:7743-8.
102. Hernandez S, Lopez-Knowles E, Lloreta J, Kogevinas M, Amoros A, Tardon A, et al. Prospective study of FGFR3 mutations as a prognostic factor in nonmuscle invasive urothelial bladder carcinomas. J Clin Oncol 2006;24:3664-71.
103. Burger M, Catto J, Van Oers J, Zwarthoff E, Hamdy FC, Meuth M, et al.[Mutation of the FGFR3 oncogene is an independent and favorable prognostic factor for tumor-specific survival in patients with urothelial carcinoma of the upper urinary tract]. Verh Dtsch Ges Pathol 2006;90:244-52.
104. Burger M, Van Der Aa MN, Van Oers JM, Brinkmann A, Van Der Kwast Th, Steyerberg EC, et al. Prediction of Progression of Non-Muscle-Invasive Bladder Cancer by WHO 1973 and 2004 Grading and by FGFR3 Mutation Status: A Prospective Study. Eur Urol 2008;54:835-44.
105. Thompson LM, Plummer S, Schalling M, Altherr MR, Gusella JF, Housman DE, et al. A gene encoding a fibroblast growth factor receptor isolated from the Huntington disease gene region of human chromosome 4. Genomics 1991;11:1133-42.
106. Plowright EE, Li Z, Bergsagel PL, Chesi M, Barber DL, Branch DR, et al. Ectopic expression of fibroblast growth factor receptor 3 promotes myeloma cell proliferation and prevents apoptosis. Blood 2000;95:992-8.
107. Knowles MA. Role of FGFR3 in urothelial cell carcinoma: biomarker and potential therapeutic target. World J Urol 2007;25:581-93.
108. Van Rhijn BW, Montironi R, Zwarthoff EC, Jobsis AC, Van Der Kwast Th. Frequent FGFR3 mutations in urothelial papilloma. J Pathol 2002;198:245-51.
109. Cappellen D, De Oliveira C, Ricol D, De Medina S, Bourdin J, Sastre-Garau X, et al. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet 1999;23:18-20.
110. Van Rhijn BW, Lurkin I, Radvanyi F, Kirkels WJ, Van Der Kwast Th, Zwarthoff EC. The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. Cancer Res 2001;61:1265-8.

111. Kimura T, Suzuki H, Ohashi T, Asano K, Kiyota H, Eto Y. The incidence of thanatophoric dysplasia mutations in FGFR3 gene is higher in low-grade or superficial bladder carcinomas. Cancer 2001; 92:2555-61.
112. Billerey C, Chopin D, Aubriot-Lorton MH, Ricol D, Gil Diez De Medina S, Van Rhijn B, et al. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am J Pathol 2001;158:1955-9.
113. Junker K, Van Oers JM, Zwarthoff EC, Kania I, Schubert J, Hartmann A. Fibroblast growth factor receptor 3 mutations in bladder tumors correlate with low frequency of chromosome alterations. Neoplasia 2008;10:1-7.
114. Junker K, Van Oers JM, Zwarthoff EC, Kania I, Schubert J, Hartmann A.[Correlation of FGFR3 mutations and chromosomal alterations in bladder cancer]. Verh Dtsch Ges Pathol 2006;90:151-8.
115. Karoui M, Hofmann-Radvanyi H, Zimmermann U, Couvelard A, Degott C, Faridoni-Laurens L, et al. No evidence of somatic FGFR3 mutation in various types of carcinoma. Oncogene 2001;20: 5059-61.
116. Naimi B, Latil A, Berthon P, Cussenot O. No evidence for fibroblast growth factor receptor 3 (FGFR-3) R248C/S249C mutations in human prostate cancer. Int J Cancer 2000;87:455-6.
117. Sibley K, Stern P, Knowles MA. Frequency of fibroblast growth factor receptor 3 mutations in sporadic tumours. Oncogene 2001;20:4416-8.
118. Hafner C, Van Oers JM, Vogt T, Landthaler M, Stoehr R, Blaszyk H, et al. Mosaicism of activating FGFR3 mutations in human skin causes epidermal nevi. J Clin Invest 2006;116:2201-07.
119. Hafner C, Van Oers JM, Hartmann A, Landthaler M, Stoehr R, Blaszyk H, et al. High frequency of FGFR3 mutations in adenoid seborrheic keratoses. J Invest Dermatol 2006;126:2404-7.
120. Hafner C, Hartmann A, Van Oers JM, Stoehr R, Zwarthoff EC, Hofstaedter F, et al. FGFR3 mutations in seborrheic keratoses are already present in flat lesions and associated with age and localization. Mod Pathol 2007;20:895-903.
121. Tsai YC, Nichols PW, Hiti AL, Williams Z, Skinner DG, Jones PA. Allelic losses of chromosomes 9, 11, and 17 in human bladder cancer. Cancer Res 1990;50:44-7.
122. Cairns P, Shaw ME, Knowles MA. Initiation of bladder cancer may involve deletion of a tumour-suppressor gene on chromosome 9. Oncogene 1993;8:1083-5.
123. Linnenbach AJ, Pressler LB, Seng BA, Kimmel BS, Tomaszewski JE, Malkowicz SB. Characterization of chromosome 9 deletions in transitional cell carcinoma by microsatellite assay. Hum Mol Genet 1993;2:1407-11.
124. Lindgren D, Liedberg F, Andersson A, Chebil G, Gudjonsson S, Borg A, et al. Molecular characterization of early-stage bladder carcinomas by expression profiles, FGFR3 mutation status, and loss of 9q. Oncogene 2006;25:2685-96.
125. Junker K, Boerner D, Schulze W, Utting M, Schubert J, Werner W. Analysis of genetic alterations in normal bladder urothelium. Urology 2003;62:1134-8.
126. Stoehr R, Zietz S, Burger M, Filbeck T, Denzinger S, Obermann EC, et al. Deletions of chromosomes 9 and 8p in histologically normal urothelium of patients with bladder cancer. Eur Urol 2005;47: 58-63.
127. Harris AL, Neal DE. Bladder cancer--field versus clonal origin. N Engl J Med 1992;326:759-61.
128. Chaturvedi V, Li L, Hodges S, Johnston D, Ro JY, Logothetis C, et al. Superimposed histologic and genetic mapping of chromosome 17 alterations in human urinary bladder neoplasia. Oncogene 1997;14:2059-70.
129. Czerniak B, Chaturvedi V, Li L, Hodges S, Johnston D, Roy JY, et al. Superimposed histologic and genetic mapping of chromosome 9 in progression of human urinary bladder neoplasia: implications for a genetic model of multistep urothelial carcinogenesis and early detection of urinary bladder cancer. Oncogene 1999;18:1185-96.
130. Pycha A, Mian C, Hofbauer J, Brossner C, Haitel A, Wiener H, et al. Multifocality of transitional cell carcinoma results from genetic instability of entire transitional epithelium. Urology 1999;53: 92-7.
131. Van Tilborg AA, De Vries A, De Bont M, Groenfeld LE, Van Der Kwast Th, Zwarthoff EC. Molecular evolution of multiple recurrent cancers of the bladder. Hum Mol Genet 2000;9:2973-80.
132. Hartmann A, Rosner U, Schlake G, Dietmaier W, Zaak D, Hofstaedter F, et al. Clonality and genetic divergence in multifocal low-grade superficial urothelial carcinoma as determined by chromosome 9 and p53 deletion analysis. Lab Invest 2000;80:709-18.

133. Catto JW, Hartmann A, Stoehr R, Bolderson E, Rehman I, Rosario DJ, et al. Multifocal urothelial cancers with the mutator phenotype are of monoclonal origin and require panurothelial treatment for tumor clearance. J Urol 2006;175:2323-30.
134. Hoglund M. Bladder cancer, a two phased disease? Semin Cancer Biol 2007;17:225-32.
135. Spruck CH, 3rd, Ohneseit PF, Gonzalez-Zulueta M, Esrig D, Miyao N, Tsai YC, et al. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res 1994;54:784-8.
136. Van Rhijn BW, Van Der Kwast Th, Vis AN, Kirkels WJ, Boeve ER, Jobsis AC, et al. FGFR3 and P53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. Cancer Res 2004;64:1911-4.
137. Tsuji M, Kojima K, Murakami Y, Kanayama H, Kagawa S. Prognostic value of Ki-67 antigen and p53 protein in urinary bladder cancer: immunohistochemical analysis of radical cystectomy specimens. Br J Urol 1997;79:367-72.
138. Van Rhijn BW, Smit M, Van Geenen D, Wijnmaalen A, Kirkels WJ, Van Der Kwast Th, et al. Surveillance with microsatellite analysis of urine in bladder cancer patients treated by radiotherapy. Eur Urol 2003;43:369-73.
139. Barbisan F, Santinelli A, Mazzucchelli R, Lopez-Beltran A, Cheng L, Scarpelli M, et al. Strong immunohistochemical expression of fibroblast growth factor receptor 3, superficial staining pattern of cytokeratin 20, and low proliferative activity define those papillary urothelial neoplasms of low malignant potential that do not recur. Cancer 2008;112:636-44.
140. Hopman AH, Kamps MA, Speel EJ, Schapers RF, Sauter G, Ramaekers FC. Identification of chromosome 9 alterations and p53 accumulation in isolated carcinoma in situ of the urinary bladder versus carcinoma in situ associated with carcinoma. Am J Pathol 2002;161:1119-25.
141. Lamy A, Gobet F, Laurent M, Blanchard F, Varin C, Moulin C, et al. Molecular profiling of bladder tumors based on the detection of FGFR3 and TP53 mutations. J Urol 2006;176:2686-9.
142. Dalesio O, Schulman CC, Sylvester R, De Pauw M, Robinson M, Denis L, et al. Prognostic factors in superficial bladder tumors. A study of the European Organization for Research on Treatment of Cancer: Genitourinary Tract Cancer Cooperative Group. J Urol 1983;129:730-3.
143. Riley GF, Potosky AL, Lubitz JD, Kessler LG. Medicare payments from diagnosis to death for elderly cancer patients by stage at diagnosis. Med Care 1995;33:828-41.
144. Schrag D, Hsieh LJ, Rabbani F, Bach PB, Herr H, Begg CB. Adherence to surveillance among patients with superficial bladder cancer. J Natl Cancer Inst 2003;95:588-97.
145. Lotan Y, Svatek RS, Sagalowsky AI. Should we screen for bladder cancer in a high-risk population?: A cost per life-year saved analysis. Cancer 2006;107:982-90.
146. Brown ML, Lipscomb J, Snyder C. The burden of illness of cancer: economic cost and quality of life. Annu Rev Public Health 2001;22:91-113.
147. Sherman AB, Koss LG, Adams SE. Interobserver and intraobserver differences in the diagnosis of urothelial cells. Comparison with classification by computer. Anal Quant Cytol 1984;6:112-20.
148. Van Der Poel HG, Debruyne FM. Can biological markers replace cystoscopy? An update. Curr Opin Urol 2001;11:503-9.
149. Simon MA, Lokeshwar VB, Soloway MS. Current bladder cancer tests: unnecessary or beneficial? Crit Rev Oncol Hematol 2003;47:91-107.
150. Van Rhijn BW. Considerations on the use of urine markers for bladder cancer. Eur Urol 2008;53: 880-1.
151. Van Rhijn BW, Van Der Poel HG, Van Der Kwast Th. Urine markers for bladder cancer surveillance: a systematic review. Eur Urol 2005;47:736-48.
152. Sylvester RJ, Van Der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 2006;49:466-5; discussion 75-7.
153. Moonen PM, Merkx GF, Peelen P, Karthaus HF, Smeets DF, Witjes JA. UroVysion compared with cytology and quantitative cytology in the surveillance of non-muscle-invasive bladder cancer. Eur Urol 2007;51:1275-80; discussion 80.
154. Mao L, Schoenberg MP, Scicchitano M, Erozan YS, Merlo A, Schwab D, et al. Molecular detection of primary bladder cancer by microsatellite analysis. Science 1996;271:659-62.

155. Mourah S, Cussenot O, Vimont V, Desgrandchamps F, Teillac P, Cochant-Priollet B, et al. Assessment of microsatellite instability in urine in the detection of transitional-cell carcinoma of the bladder. Int J Cancer 1998;79:629-33.
156. Utting M, Werner W, Dahse R, Schubert J, Junker K. Microsatellite analysis of free tumor DNA in urine, serum, and plasma of patients: a minimally invasive method for the detection of bladder cancer. Clin Cancer Res 2002;8:35-40.
157. Van Rhijn BW, Lurkin I, Kirkels WJ, Van Der Kwast Th, Zwarthoff EC. Microsatellite analysis--DNA test in urine competes with cystoscopy in follow-up of superficial bladder carcinoma: a phase II trial. Cancer 2001;92:768-75.
158. Zhang J, Zheng S, Fan Z, Gao Y, Di X, Wang D, et al. A comparison between microsatellite analysis and cytology of urine for the detection of bladder cancer. Cancer Lett 2001;172:55-8.
159. Lutzeyer W, Rubben H, Dahm H. Prognostic parameters in superficial bladder cancer: an analysis of 315 cases. J Urol 1982;127:250-2.
160. Heney NM, Ahmed S, Flanagan MJ, Frable W, Corder MP, Hafermann MD, et al. Superficial bladder cancer: progression and recurrence. J Urol 1983;130:1083-6.
161. Allard P, Bernard P, Fradet Y, Tetu B. The early clinical course of primary Ta and T1 bladder cancer: a proposed prognostic index. Br J Urol 1998;81:692-8.
162. Smith G, Elton RA, Beynon LL, Newsam JE, Chisholm GD, Hargreave TB. Prognostic significance of biopsy results of normal-looking mucosa in cases of superficial bladder cancer. Br J Urol 1983;55: 665-9.
163. Shinka T, Hirano A, Uekado Y, Ohkawa T. Clinical study of prognostic factors of superficial bladder cancer treated with intravesical bacillus Calmette-Guerin. Br J Urol 1990;66:35-9.
164. Kiemeney LA, Witjes JA, Heijbroek RP, Debruyne FM, Verbeek AL. Dysplasia in normal-looking urothelium increases the risk of tumour progression in primary superficial bladder cancer. Eur J Cancer 1994;30A:1621-5.
165. Mulders PF, Meyden AP, Doesburg WH, Oosterhof GO, Debruyne FM. Prognostic factors in pTa-pT1 superficial bladder tumours treated with intravesical instillations. The Dutch South-Eastern Urological Collaborative Group. Br J Urol 1994;73:403-8.
166. Kondo T, Onitsuka S, Ryoji O, Kihara T, Goto Y, Satoh T, et al. Analysis of prognostic factors related to primary superficial bladder cancer tumor recurrence in prophylactic intravesical epirubicin therapy. Int J Urol 1999;6:178-83.
167. Kiemeney LA, Witjes JA, Heijbroek RP, Verbeek AL, Debruyne FM. Predictability of recurrent and progressive disease in individual patients with primary superficial bladder cancer. J Urol 1993; 150:60-4.
168. Flamm J, Havelec L. Factors affecting survival in primary superficial bladder cancer. Eur Urol 1990; 17:113-8.
169. Lipponen PK, Eskelinen M, Jauhiainen K, Terho R, Harju E. Clinical prognostic factors in transitional cell cancer of the bladder. Urol Int 1993;50:192-7.
170. Zieger K, Wolf H, Olsen PR, Hojgaard K. Long-term survival of patients with bladder tumours: the significance of risk factors. Br J Urol 1998;82:667-72.
171. Witjes JA, Kiemeney LA, Oosterhof GO, Debruyne FM. Prognostic factors in superficial bladder cancer. A review. Eur Urol 1992;21:89-97.
172. Aso Y, Anderson L, Soloway M, Bouffioux C, Chisholm G, Debruyne F, et al. Prognostic factors in superficial bladder cancer. Prog Clin Biol Res 1986;221:257-69.
173. Oosterlinck W, Lobel B, Jakse G, Malmstrom PU, Stockle M, Sternberg C. Guidelines on bladder cancer. Eur Urol 2002;41:105-12.
174. Pfister C, Moore L, Allard P, Larue H, Lacombe L, Tetu B, et al. Predictive value of cell cycle markers p53, MDM2, p21, and Ki-67 in superficial bladder tumor recurrence. Clin Cancer Res 1999;5:4079-84.
175. Stavropoulos NE, Filiadis I, Ioachim E, Hastazeris K, Tsimaris I, Kalogeras D, et al. Prognostic significance of p53, bcl-2 and Ki-67 in high risk superficial bladder cancer. Anticancer Res 2002;22: 3759-64.
176. Knowles MA, Elder PA, Williamson M, Cairns JP, Shaw ME, Law MG. Allelotype of human bladder cancer. Cancer Res 1994;54:531-8.
177. Knowles MA. Tumor suppressor loci in bladder cancer. Front Biosci 2007;12:2233-51.

178. Tomlinson DC, Baldo O, Harnden P, Knowles MA. FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer. J Pathol 2007;213:91-8.
179. Shariat SF, Karam JA, Lerner SP. Molecular markers in bladder cancer. Curr Opin Urol 2008;18:1-8.
180. Van Oers JM, Adam C, Denzinger S, Stoehr R, Bertz S, Zaak D, et al. Chromosome 9 deletions are more frequent than FGFR3 mutations in flat urothelial hyperplasias of the bladder. Int J Cancer 2006;119:1212-5.
181. Birkhahn M, Mitra AP, Cote RJ. Molecular markers for bladder cancer: the road to a multimarker approach. Expert Rev Anticancer Ther 2007;7:1717-27.
182. Fitzpatrick JM, West AB, Butler MR, Lane V, O'Flynn JD. Superficial bladder tumors (stage pTa, grades 1 and 2): the importance of recurrence pattern following initial resection. J Urol 1986;135: 920-2.
183. Epstein JI, Carmichael MJ, Partin AW, Walsh PC. Small high-grade adenocarcinoma of the prostate in radical prostatectomy specimens performed for nonpalpable disease: pathogenetic and clinical implications. J Urol 1994;151:1587-92.
184. Larsson P, Wijkstrom H, Thorstenson A, Adolfsson J, Norming U, Wiklund P, et al. A population-based study of 538 patients with newly detected urinary bladder neoplasms followed during 5 years. Scand J Urol Nephrol 2003;37:195-201.
185. Malmstrom PU, Busch C, Norlen BJ. Recurrence, progression and survival in bladder cancer. A retrospective analysis of 232 patients with greater than or equal to 5-year follow-up. Scand J Urol Nephrol 1987;21:185-95.
186. Pich A, Chiusa L, Formiconi A, Galliano D, Bortolin P, Navone R. Biologic differences between noninvasive papillary urothelial neoplasms of low malignant potential and low-grade (grade 1) papillary carcinomas of the bladder. Am J Surg Pathol 2001;25:1528-33.
187. Jordan AM, Weingarten J, Murphy WM. Transitional cell neoplasms of the urinary bladder. Can biologic potential be predicted from histologic grading? Cancer 1987;60:2766-74.
188. Lebret T, Bohin D, Kassardjian Z, Herve JM, Molinie V, Barre P, et al. Recurrence, progression and success in stage Ta grade 3 bladder tumors treated with low dose bacillus Calmette-Guerin instillations. J Urol 2000;163:63-7.
189. Cheng L, Cheville JC, Neumann RM, Leibovich BC, Egan KS, Spotts BE, et al. Survival of patients with carcinoma in situ of the urinary bladder. Cancer 1999;85:2469-74.
190. Esrig D, Freeman JA, Elmajian DA, Stein JP, Chen SC, Groshen S, et al. Transitional cell carcinoma involving the prostate with a proposed staging classification for stromal invasion. J Urol 1996;156: 1071-6.
191. Bol MG, Baak JP, Buhr-Wildhagen S, Kruse AJ, Kjellevold KH, Janssen EA, et al. Reproducibility and prognostic variability of grade and lamina propria invasion in stages Ta, T1 urothelial carcinoma of the bladder. J Urol 2003;169:1291-4.
192. Cheng L, Weaver AL, Neumann RM, Scherer BG, Bostwick DG. Substaging of T1 bladder carcinoma based on the depth of invasion as measured by micrometer: A new proposal. Cancer 1999;86: 1035-43.
193. Platz CE, Cohen MB, Jones MP, Olson DB, Lynch CF. Is microstaging of early invasive cancer of the urinary bladder possible or useful? Mod Pathol 1996;9:1035-9.
194. Holmang S, Hedelin H, Anderstrom C, Holmberg E, Johansson SL. The importance of the depth of invasion in stage T1 bladder carcinoma: a prospective cohort study. J Urol 1997;157:800-3; discussion 04.
195. Lapham RL, Grignon D, Ro JY. Pathologic prognostic parameters in bladder urothelial biopsy, transurethral resection, and cystectomy specimens. Semin Diagn Pathol 1997;14:109-22.
196. Bakkar AA, Wallerand H, Radvanyi F, Lahaye JB, Pissard S, Lecerf L, et al. FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder. Cancer Res 2003;63:8108-12.
197. Hickman ES, Moroni MC, Helin K. The role of p53 and pRB in apoptosis and cancer. Curr Opin Genet Dev 2002;12:60-6.
198. Lane DP. Cancer. p53, guardian of the genome. Nature 1992;358:15-6.
199. Esrig D, Elmajian D, Groshen S, Freeman JA, Stein JP, Chen SC, et al. Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med 1994;331:1259-64.

200. Serth J, Kuczyk MA, Bokemeyer C, Hervatin C, Nafe R, Tan HK, et al. p53 immunohistochemistry as an independent prognostic factor for superficial transitional cell carcinoma of the bladder. Br J Cancer 1995;71:201-5.
201. Knowles MA. What we could do now: molecular pathology of bladder cancer. Mol Pathol 2001;54: 215-21.
202. Primdahl H, Von Der Maase H, Christensen M, Wolf H, Orntoft TF. Allelic deletions of cell growth regulators during progression of bladder cancer. Cancer Res 2000;60:6623-9.
203. Duchrow M, Schluter C, Wohlenberg C, Flad HD, Gerdes J. Molecular characterization of the gene locus of the human cell proliferation-associated nuclear protein defined by monoclonal antibody Ki-67. Cell Prolif 1996;29:1-12.
204. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. Int J Cancer 1983;31:13-20.
205. Stein JP, Grossfeld GD, Ginsberg DA, Esrig D, Freeman JA, Figueroa AJ, et al. Prognostic markers in bladder cancer: a contemporary review of the literature. J Urol 1998;160:645-59.
206. Quintero A, Alvarez-Kindelan J, Luque RJ, Gonzalez-Campora R, Requena MJ, Montironi R, et al. Ki-67 MIB1 labelling index and the prognosis of primary TaT1 urothelial cell carcinoma of the bladder. J Clin Pathol 2006;59:83-8.
207. Asakura T, Takano Y, Iki M, Suwa Y, Noguchi S, Kubota Y, et al. Prognostic value of Ki-67 for recurrence and progression of superficial bladder cancer. J Urol 1997;158:385-8.
208. Lopez-Beltran A, Luque RJ, Alvarez-Kindelan J, Quintero A, Merlo F, Requena MJ, et al. Prognostic factors in survival of patients with stage Ta and T1 bladder urothelial tumors: the role of G1-S modulators (p53, p21Waf1, p27Kip1, cyclin D1, and cyclin D3), proliferation index, and clinicopathologic parameters. Am J Clin Pathol 2004;122:444-52.
209. Christoph F, Weikert S, Wolff I, Schostak M, Tabiti K, Muller M, et al. Urinary cytokeratin 20 mRNA expression has the potential to predict recurrence in superficial transitional cell carcinoma of the bladder. Cancer Lett 2007;245:121-6.
210. Parker DC, Folpe AL, Bell J, Oliva E, Young RH, Cohen C, et al. Potential utility of uroplakin III, thrombomodulin, high molecular weight cytokeratin, and cytokeratin 20 in noninvasive, invasive, and metastatic urothelial (transitional cell) carcinomas. Am J Surg Pathol 2003;27:1-10.
211. Harnden P, Mahmood N, Southgate J. Expression of cytokeratin 20 redefines urothelial papillomas of the bladder. Lancet 1999;353:974-7.
212. Gerharz EW, Mansson A, Mansson W. Quality of life in patients with bladder cancer. Urol Oncol 2005;23:201-7.
213. Hardt J, Filipas D, Hohenfellner R, Egle UT. Quality of life in patients with bladder carcinoma after cystectomy: first results of a prospective study. Qual Life Res 2000;9:1-12.
214. Kaasa S, Loge JH. Quality-of-life assessment in palliative care. Lancet Oncol 2002;3:175-82.
215. Ringdal GI, Ringdal K. A follow-up study of the quality of life in cancer patients with different prognoses. Qual Life Res 2000;9:65-73.
216. Elzevier HW, Nieuwkamer BB, Pelger RC, Lycklama à Nijeholt AA. Female sexual function and activity following cystectomy and continent urinary tract diversion for benign indications: a clinical pilot study and review of literature. J Sex Med 2007;4:406-16.
217. Kortmann BB, Sonke GS, D'Ancona FC, Floratos DL, Debruyne FM, De La Rosette JJ. The tolerability of urodynamic studies and flexible cysto-urethroscopy used in the assessment of men with lower urinary tract symptoms. BJU Int 1999;84:449-53.
218. Denholm SW, Conn IG, Newsam JE, Chisholm GD. Morbidity following cystoscopy: comparison of flexible and rigid techniques. Br J Urol 1990;66:152-4.
219. Taghizadeh AK, El Madani A, Gard PR, Li CY, Thomas PJ, Denyer SP. When does it hurt? Pain during flexible cystoscopy in men. Urol Int 2006;76:301-3.
220. Muezzinoglu T, Ceylan Y, Temeltas G, Lekili M, Buyuksu C. Evaluation of pain caused by urethrocystoscopy in patients with superficial bladder cancer: a perspective of quality of life. Onkologie 2005;28:260-4.
221. Herr HW, Schneider M. Outpatient flexible cystoscopy in men: a randomized study of patient tolerance. J Urol 2001;165:1971-2.
222. Stav K, Leibovici D, Goren E, Livshitz A, Siegel YI, Lindner A, et al. Adverse effects of cystoscopy and its impact on patients' quality of life and sexual performance. Isr Med Assoc J 2004;6:474-8.

223. Vriesema JL, Poucki MH, Kiemeney LA, Witjes JA. Patient opinion of urinary tests versus flexible urethrocystoscopy in follow-up examination for superficial bladder cancer: a utility analysis. Urology 2000;56:793-7.
224. Yossepowitch O, Herr HW, Donat SM. Use of urinary biomarkers for bladder cancer surveillance: patient perspectives. J Urol 2007;177:1277-82; discussion 82.
225. Cooksley CD, Avritscher EB, Grossman HB, Sabichi AL, Dinney CP, Pettaway C, et al. Clinical model of cost of bladder cancer in the elderly. Urology 2008;71:519-25.
226. Avritscher EB, Cooksley CD, Grossman HB, Sabichi AL, Hamblin L, Dinney CP, et al. Clinical model of lifetime cost of treating bladder cancer and associated complications. Urology 2006;68:549-53.

CHAPTER 2

Outline of the thesis

Outline of the thesis

In an ideal health care scenario the four aspects of bladder cancer patient care (that is diagnosis and risk stratification of patients, surveillance, optimal quality of life and societal impact of the management of bladder cancer) would be integrated in such a way that an optimal management can be offered to patients with NMI UC. This thesis on surveillance of patients with non-muscle invasive bladder cancer attempts to analyse these four aspects as reflected in the contents of its parts**: Part I** General introduction provides a background on epidemiology, diagnosis, treatment and biology of bladder cancer, **Part II** is reporting our studies on the clinical relevance of conventional and novel prognostic factors for bladder cancer behaviour, **Part III** is devoted to the analysis of an alternative surveillance scheme in patients with non-muscle invasive bladder cancers, **Part IV** is reporting on the impact of surveillance on quality of life, **Part V** analyses the economic impact of bladder cancer surveillance with an alternative urine test and **Part VI** discusses the results of the studies of this thesis. **Parts III-V** report specifically the outcomes of a randomized multicenter prospective trial on the cost-effectiveness of the follow-up of patients with non-muscle invasive bladder cancer (CEFUB-trial) conducted in the region surrounding Rotterdam.

In more detail, **Part I, chapter 1** of the thesis gives the necessary background of this thesis, describing the molecular biology and cancer genetics of bladder cancer underlying the diagnosis and prognosis in bladder cancer.

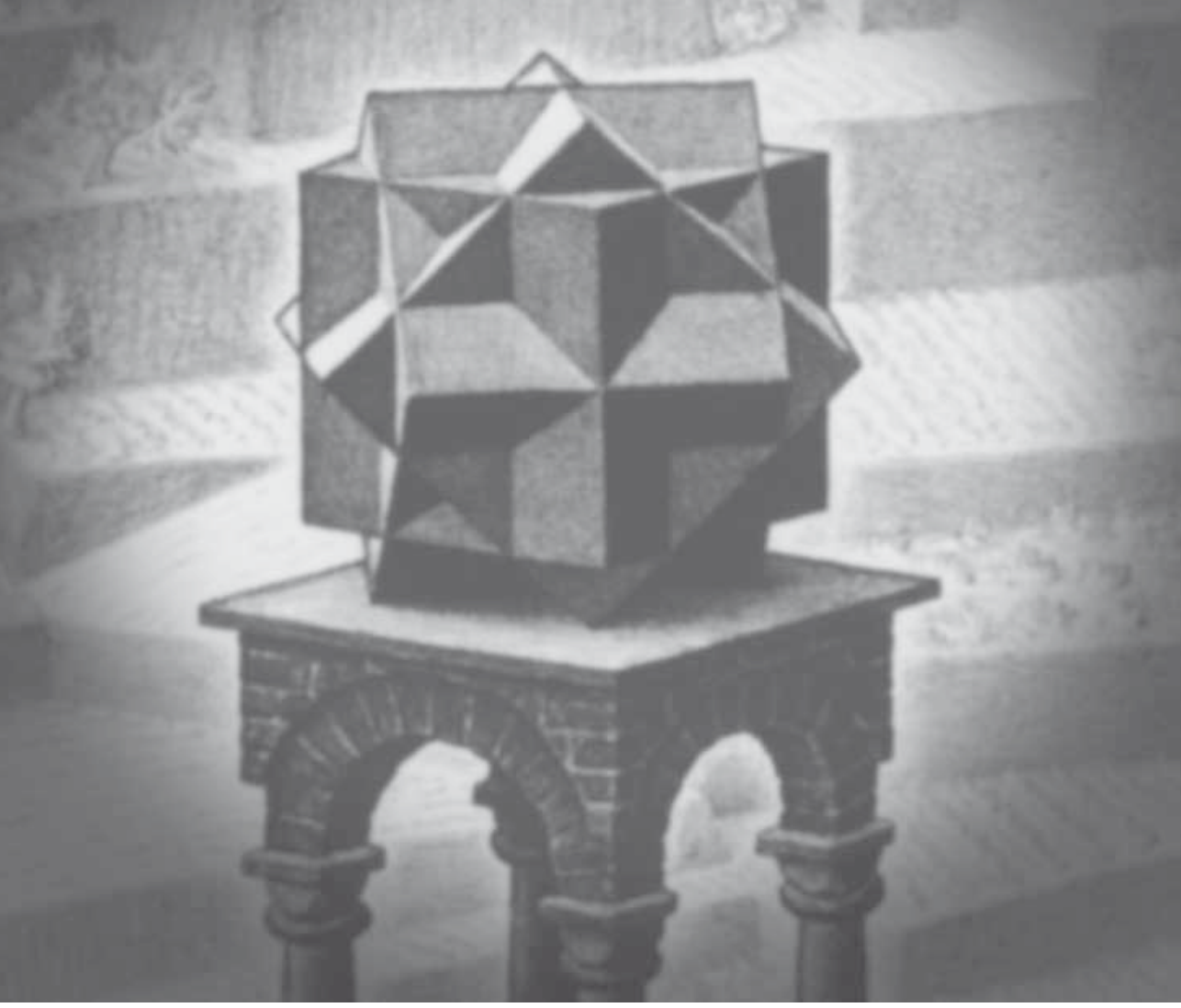
Part II aims to answer the question how to predict bladder cancer behaviour by tissue biomarkers in order to achieve optimal risk stratification. Knowledge of the biologic behaviour of newly diagnosed cancer might enable the identification of subgroups of patients to offer a tailor-made surveillance scheme. **Chapters 3–5** comprise **part II** of the thesis, analyzing conventional and novel prognosticators for NMI UC. A meta-analysis of 5021 patients with bladder cancer described in **chapter 3** analyses the prognostic value of conventional clinical and pathological parameters. To individualize surveillance strategies it is necessary to distinguish in a meaningful way between high and low risk patients in the group of non-muscle invasive bladder cancer patients (NMI UC). By the introduction of the WHO 2004 classification system of bladder cancers a change in grading of bladder cancers has been introduced, but some controversy remained with regard to the clinical impact of this new grading system as compared the older WHO 1973 system. Further, there is evidence that novel prognostic factors like *FGFR3* gene mutation status and immuno-histochemical staining for a number of biomarkers (e.g. Ki-67 labeling index, cytokeratin 20 staining pattern) may be of additional value to improve patient stratification. In **Chapter 4** we describe in a prospective study involving multiple contributing hospitals the prognostic impact of two grading systems (WHO 1973 and 2004) together with the biomarkers *FGFR3* mutation status, CK-20 expression pattern and Ki-67 labeling index. The possibility to subdivide the subset of NMI UC that invades into the sub-epithelial connective tissue (pT1) at first presentation is of

great clinical interest. The behaviour of these pT1 UC is very unpredictable and they seem to display a considerable biological and molecular heterogeneity. In **chapter 5** we propose a simplified pathological substaging system for pT1 UC, we related this novel substaging method to *FGFR3* mutation status and tumour grade and we investigated whether we were able to improve the risk stratification of pT1 UC by any of these markers. Surveillance (**Part III**) of patients with NMI UC after diagnosis is currently done by an intensive scheme of cystoscopic examinations. Because cystoscopy is generally considered a burdensome and costly method new preferably non-invasive methods have been developed. ***Can we improve surveillance of bladder cancer?*** Several studies suggested that microsatellite analysis (MA) of voided urine samples for detection of cells with loss of heterozygosity (LOH) might offer a reliable substitute for cystoscopy. Further, MA may also be able to detect upper urinary tract UC. For a new method to compete with cystoscopy it needs to be feasible in routine clinical practice: highly reproducible, accurate and at acceptable costs. For this reason we engaged on a prospective randomized multicenter trial on patients with low-grade (WHO 1973 grade 1 and 2), low stage (pTa, pT1) bladder cancer, comparing a conventional follow-up scheme by cystoscopy alone to a follow-up scheme in which part of the cystoscopies was replaced by MA (**chapter 6**). The findings of this randomized trial prompted us to further scrutinize the view that cystoscopy represents the gold standard for detection of UC recurrences in patients with NMI UC (**chapter 7**).

How does surveillance affect patients with NMI UC? The influence of surveillance of bladder cancer on their quality of life is the topic of chapters 8 and 9 of **Part IV**. We aimed to quantify the experienced burden of patients at one week after cystoscopy in order to verify the generally assumed heavy impact of cystoscopy on the patients' perceived quality of life (**chapter 8**). In a small side study we also examined to what extent sexual dysfunction occurs in patients with NMI UC (**chapter 9**).

What are the effects versus the costs in surveillance if cystoscopy is partly replaced by the MA urinary test? To implement a new method in a routine clinical practice, patients, clinicians as well as public health care providers must be convinced that the new scheme is more cost-effective than the traditional surveillance scheme. Cost-effectiveness includes economic costs, quality of life and effects of the method on the health status of the patient. **Chapter 10** of **part V** reports on an analysis of cost-effectiveness of the surveillance of patients with NMI UC in which cystoscopy is partly replaced by MA. This analysis tries to determine how high the costs of an alternative urine test may be in relationship to its accuracy and effects on health status in order to be implemented successfully.

The final **part VI** comprises **chapter 11 and 12.** In the former chapter the results of our study are discussed and an outline of future studies aimed at the improvement of surveillance for patients with NMI UC is provided. The latter chapter summarizes the content of the studies described in this thesis.



PART II

BLADDER CANCER BIOLOGY

CHAPTER 3

Prognostic factors: a meta-analysis

CHAPTER 4

Prediction of progression by WHO 1973 and 2004 grading and by *FGFR3* status

CHAPTER 5

A new system for substaging pT1

CHAPTER 3

Clinical and Pathological Prognostic Factors for Recurrence, Progression and Mortality in non-muscle invasive Bladder Cancer: a Meta-analysis

Madelon NM van der Aa *
Mustafa Dönmez *
Marinus JC Eijkemans
Theo H van der Kwast
Ellen C Zwarthoff
Ewout W Steyerberg

** contributed equally*

Abstract

We aimed to quantify the importance of clinical and pathological prognostic factors in the outcome of non-muscle invasive bladder cancer (NMI UC) based on current literature. We identified 14 publications published between 1980 and 2004 that reported on the association between clinical and pathological prognostic factors for tumour recurrence, tumour progression and overall mortality after transurethral resection of a NMI UC tumour. Relative risks (RR) were estimated using meta-analytic techniques to combine results of separate studies. In total, 2536 of 5021 patients had a tumour recurrence, 317 of 3313 had tumour progression and 531 of 1548 died. The strongest prognostic factor for tumour recurrence was the result of the first 3-monthly cystoscopy (RR, 2.2; 95% CI, 1.8-2.7). The strongest prognostic factor for tumour progression was grade 3 compared to grade 1 (RR, 6.7; 95% CI 4.7-9.5). The presence of CIS had a strong effect on tumour progression (RR, 4.4; 95% CI 3.4-5.5) and on the overall mortality (RR, 4.0; 95% CI 2.4-6.5). Tumour shape, grade 3 and patient age also had strong effects on survival. Clinical and pathological prognostic factors could not predict tumour recurrence, but showed strong associations with tumour progression and overall mortality. To individualize surveillance strategies emphasis should be on the search for novel prognostic factors that can reliably predict recurrence.

Introduction

Bladder cancer is the fourth most common malignancy in men and the eight most common in women[1]. The majority of the urinary bladder cancers are non-muscle invasive urothelial carcinomas (NMI UC)[1]. The muscle- invasive UC have a significantly worse prognosis[2]. A major problem of NMI UC is that it recurs in up to 70% of the cases after a transurethral resection (TUR)[3]. Approximately 10-30% of these recurrences will progress to a more malignant form[4-7], with a 5-year survival rate after cystectomy of 45%[2]. Therefore it is necessary for the patients to be monitored at regular intervals. Follow up consists worldwide of schemes of cystoscopy, the gold standard, and cytology.

Because of the high costs related to surveillance of patients with a NMI UC, the benefit of maintaining a fixed follow up scheme for all patients, irrespective of their individual risk, is questionable[8, 9]. A first step towards individualizing the follow up policy of NMI UC is the search for prognostic factors to classify patients into different risk groups. Much research has been conducted to identify and quantify prognostic factors for the risk of recurrence[5, 8, 10-18], risk of progression[5, 14, 16, 18] and mortality risk[16, 19-21] in NMI UC patients. In addition several reviews have qualitatively summarized studies on prognostic factors[22, 23]. These reviews however were not carried out systematically, and did not include formal statistical techniques to appropriately combine results from individual studies.

We therefore aimed to perform a systematic review to quantify the strength of prognostic factors more accurately. More objective prognostic effects can be obtained by analyzing all available studies, including those reporting no significant effects. Also, by consideration of larger numbers of patients, effects can be estimated more precisely. Systematic reviews have therefore been promoted as at the core of evidence-based medicine[24].

Patients and Materials

Study Identification

We searched the MEDLINE database from 1980 to 2004 to identify English-language studies on clinical and pathological prognostic factors for tumour recurrence, tumour progression and mortality in NMI UC (transitional) of the urinary bladder. Search words were "prognostic factors" and "Superficial bladder cancer" and "non-muscle invasive bladder cancer". Relevant references in articles were also considered.

Studies were included if the association between any of the three above mentioned end-points and the clinical and pathological characteristics were quantified, or if it was possible to calculate relative risks from the reported data. In order to limit publication bias, studies were excluded if it was not possible to calculate the relative risks of non-significant prognostic factors from the reported data. Publication bias might arise when a positive or statistically

significant association is more likely to be reported than a negative or statistically non-significant association. When two studies included patients from the same center with overlapping time periods, we included the study with the largest population or with the longest follow up period. Studies had to have patients with primary and/or recurrent NMI bladder cancer to be included for analysis. We defined NMI UC as tumour extension limited to the mucosa (stage Ta) or limited to the lamina propria (stage T1). Primary carcinoma in situ (CIS) without other disease was not considered to be a NMI tumour. Studies on patients with NMI UC tumours located in the upper urinary tract were excluded. We also excluded studies with patients likely to have a worse prognosis, so who had received chemotherapy, radiotherapy or had undergone a cystectomy prior to the start of the study.

Clinical and Pathological Characteristics

We studied the following clinical and pathological characteristics: patient gender, patient age, stage of the tumour, grade of the tumour, multiplicity of tumours, presence of dysplasia, presence of CIS, tumour size, location of the tumour, the shape of the tumour, disease status, result of cystoscopy at 3 months after TUR. These prognostic factors were chosen since they were considered in more than one study.

The stage of the tumour was considered when it was determined according to the tumour-node-metastasis system classification of '78[25]: Stage pT1 was compared to pTa. All of the studies classified the grade of the tumour according to the WHO classification system of '73[26]. One study used both a local and reference pathologist to stage and grade the tumours[12]. Only the data from the reference pathologist was analyzed. Multiplicity was defined as the presence of multiple tumours at diagnosis. Multiple tumours were compared to solitary tumours. Results from a study that compared patients with 6 or less tumours with patients with 7 or more tumours were therefore not analyzed[16]. The presence of dysplasia was compared to normal urothelium. One study compared normal urothelium to mild/moderate dysplasia and to severe dysplasia[5]. Mild, moderate and severe dysplasia were all considered as the presence of dysplasia. Different cutoff values were chosen for the size of the tumour. One study used a cutoff value of 5 cm[11], two studies used a cutoff value of 2 cm[15, 17], while the other studies used a cut-off of 3 cm[8, 10, 16, 18] and 2.5 cm[12]. We assessed two cutoff values, namely 2 cm for the two studies that used a cutoff value of 2 cm and 3 cm for the other studies that used a cutoff value of 2.5-3 cm. Cutoff values of 60 and 65 years were considered for patient age[14, 1615, 17] . We assessed the involvement of two locations of the bladder wall in tumour growth, the posterior wall and the trigone, since only these tumour locations were studied in more than one study. For the disease status a recurrent tumour was compared to a primary tumour. The first 3-monthly cystoscopy after diagnosis was either positive or negative. A positive result was defined as the presence of a recurrence and/or a progression of the tumour. For the shape of the tumour, papillary tumours were compared to tumours of other shape.

Endpoints

Three endpoints were analyzed, namely: tumour recurrence, tumour progression and overall mortality. For the risk of first tumour recurrence, most studies[4, 5, 11, 12, 14-17] used time to first tumour recurrence or the disease free interval as a measure. Three other studies[8, 10, 13] reported the recurrence rate. Tumour progression was defined as a shift to stage T2 or worse and/or the development of regional and/or distant metastases. Four studies[5, 14, 16, 18] were included for the analysis of tumour progression. Time to tumour progression or disease free interval was used as a measure for tumour progression. Survival curves were used in all studies[16, 19-21] to assess the mortality risk.

Statistical Analysis

For each prognostic factor we required relative risks and standard errors. These were sometimes directly available in the reports. Alternatively, when Kaplan-Meier curves had been reported, we scanned and enlarged published curves in a graphical computer package. We measured the heights of these curves and calculated corresponding probabilities of the events, from which relative risks were derived. The standard error of each study was estimated based on the number of patients with an endpoint. Weights were obtained by taking the inverse of the square of the standard error. These weights were used to calculate pooled relative risks (RR) for the prognostic factors, using standard meta-analytical methods[27]. Heterogeneity between studies was assessed graphically and judged satisfactory. We also report on the fraction of patients with a prognostic characteristic, since this also determines its clinical relevance. If a characteristic, for example, has a strong effect (i.e. high relative risk) a low occurrence may make it of rather limited relevance.

Results

We included fourteen studies (Table 3.1), with eleven studies reporting on tumour recurrence[5, 8, 10-18], four studies on tumour progression[5, 14, 16, 18] and four studies on the overall mortality[16, 19-21]. The total numbers of patients were respectively 5021, 3313 and 1548 for recurrence, progression and overall mortality. The reported median follow up periods ranged from 27 to 48 months for recurrence and 39 to 48 months for progression. The maximum follow up period for overall mortality ranged from 5 to 20 years (median follow up periods were not reported). A total of 2536 of 5021 patients had a recurrence, 317 of 3313 patients had progression and 531 of 1548 patients died. The follow up policy varied between the different studies (Table 3.2). Most started with 3 monthly intervals during the first years, but the frequency differed especially during later years.

Table 3.1

Recurrence

First author (ref)	Year	N	Study type	Acrual period	Patients with recurrence	Considered prognostic factors	Significant prognostic factors
Dalesio (10)	1983	308	rct	1975-1978	202	ag, gr, mu, re, si, tr, ts	mu, re, si, tr*
Heney (5)	1983	243	rct	1974-1977	133	gr, mb, mu, si, st, ur	gr, mb, mu, si, st, ur
Smith (11)	1983	112	cohort	1978-1981	59	gr, mb, mu, si, st	mb
Parmar (12)	1989	305	rct	1981-1986	143	cy, gr, lo, mu, si, st	cy, mu*
Shinka (13)	1990	198	cohort	1986-1988**	60	gr, mb, mu, pp, st	gr, mu, st
Kiemeney (14)	1994	1001	cohort	1983-1989	513	ag, ge, gr, mb, mu, nlo, st	mb, mu, nlo, st*
Mulders (15)	1994	371	rct	1987-1990	138	ag, ge, gr, lo, mu, si, st, ts	lo, mu*
Kurth (16)	1995	576	rct	1979-1987**	311	ag, ge, cy, gr, lo, mu, re, si, st, td, ts	gr, lo, re, si*
Allard (8)	1998	333	cohort	1990-1995**	230	ag, ge, gr, lo, mb, mu, si, sm, st	gr, mu, si, st*
Kondo (17)	1999	45	cohort	1989-1997	13	ag, ge, gr, mb, mu, sh, si, st	ag, sh, si*
Millan-Rodriguez (18)	2000	1529	cohort	1968-1996	734	gr, mb, mu, si, st, tr	mb, mu, si, tr*
Total	**1983-2000**	**5021**			**2536**		

Progression

First author (ref)	Year	N	Study type	Acrual period	Patients with progression	Considered prognostic factors	Significant prognostic factors
Heney (5)	1983	207	rct	1974-1977	25	gr, mb, si, st	gr, mb, si, st
Kiemeney (14)	1994	1001	cohort	1983-1989	102	ag, ge, gr, mb, mu, nlo, st	ag, gr, mb, mu*
Kurth (16)	1995	576	rct	1979-1987**	75	ag, ge, cy, gr, lo, mu, re, si, st, td, ts	cy, ge, gr, lo, re, si*
Millan-Rodriguez (18)	2000	1529	cohort	1968-1996	115	gr, mb, mu, si, st, tr	gr, mb, mu, si, tr*
Total	**1983-2000**	**3313**			**317**		

Mortality

First author (ref)	Year	N	Study type	Acrual period	Patients died	Considered prognostic factors	Significant prognostic factors
Flamm (19)	1990	345	cohort	1971-1982	40	ag, ge, gr, lo, mb, mu, rw, st, tai	gr, mb, tai*
Lipponen (20)	1993	272	cohort	1965-1991	83	ag, ge, gr, sh, st	ag, gr*
Kurth (16)	1995	576	rct	1979-1987**	127	ag, ge, cy, gr, lo, mu, re, si, st, td	ag, ge, re, si*
Zieger (21)	1998	355	cohort	1976-1984	281	gr, mb, mu, sh, si, st	gr, si, st*
Total	**1990-1998**	**1548**			**531**		

*prognostic factors found significant after multivariable analysis

**study period estimated from the reported longest follow-up period

Abbreviations: ag, age; cy, 3 months cystoscopy result; ge, gender; gr, tumour grade; lo, tumour location; mb, mucosal biopsy; mu, multiplicity; nlo, number of tumour locations; pp, purified protein derivative; rct, randomized clinical trial; re, prior recurrence rate; rw, resection weight; sh, tumour shape; si, tumour size; sm, smoking status; st, tumour stage; tai, tumour associated inflammation; td, time from diagnosis; tr, treatment; ts, tumour status; ur, urinary cytology.

Tumour recurrence

Multiplicity was considered in all eleven studies[5, 8, 10-18] and was found statistically significant in eight[5, 8, 10, 12-15, 18]. Tumour stage, tumour grade and tumour size were considered eleven[5, 8, 10-18], eleven[5, 8, 10-18] and nine times[5, 8, 10-12, 15-18], respectively, and were found significant four[5, 8, 13, 14], four[5, 8, 13, 16] and six times[5, 8, 10, 16-18], respectively (Table 3.1).

The strongest prognostic factor for tumour recurrence was the result of cystoscopy after 3 months (pooled RR, 2.2; 95% CI, 1.8-2.7, Table 3.3a). Smaller effects were found for disease status (RR 1.5; 95% CI, 1.3-1.8), trigone involvement (RR 1.5; 95% CI, 1.2-1.8), multiplicity (RR 1.4; 95% CI 1.3-1.6), tumour size (3 cm cutoff value, RR 1.4; 95% CI 1.2-1.7) and grade of disease (grade 3 compared to grade 1, RR 1.4; 95% CI 1.2-1.6). Patient age and patient gender did not have a prognostic value for tumour recurrence.

Table 3.2

Study references	Follow up policy
8,17,19-20	3 monthly first two years, 6 monthly thereafter
10,16	3 monthly first year, 4 monthly second year, 6 monthly thereafter
12,14	3 monthly first year, 6 monthly second year, yearly thereafter
13,15	3 monthly for two to three years
18	4 monthly for first two years, 6 monthly thereafter
21	4 monthly for five years

Surveillance management regarding cystoscopic examination in the 14 reviewed studies

Table 3.3a

Prognostic factor	Study references	Total patients	Patients with characteristic (%)	RR	95 % CI
Stage					
T1 v Ta	5,8,11-18	4693	2029 (43)	1.2	1.1-1.3
Grade					
2 v 1	5,8,11-14,16-18	3507	2143 (61)	1.2	1.1-1.4
3 v 1	5,8,11-14,16-18	2155	791 (37)	1.4	1.2-1.6
2-3 v 1	5,8,10-14,16-18	4298	2937 (68)	1.3	1.2-1.4
3 v 1/2	5,11-18	4336	823 (19)	1.1	1.0-1.2
Multiplicity					
multiple v solitary	5,8,10,12-15,17-18	3981	1487 (37)	1.4	1.3-1.6
Dysplasia presence					
dysplasia v normal	5,8,11,14	497	135 (27)	1.3	1.0-1.8
CIS presence					
CIS v without CIS	8,11,13-14,18	1332	95 (7)	1.2	0.9-1.6
Size (cm)					
>3 v <3	8,10,12,16,18	1369	480 (35)	1.4	1.2-1.7
>2 v <2	15,17	389	225 (58)	1.2	0.9-1.7
Gender					
female v male	8,14-17	2531	502 (20)	1.0	0.9-1.1
Posterior wall involvement					
yes v no	8,12,15-16	1446	822 (57)	1.3	1.1-1.5
Trigone involvement					
yes v no	8,15-16	1141	490 (43)	1.5	1.2-1.8
Age					
>60 v <60	14,16	1577	1155 (73)	1.0	0.8-1.1
>65 v <65	15,17	416	205 (49)	0.9	0.6-1.2
Disease status					
recurrent v primary	10,15-16	1253	557 (44)	1.5	1.3-1.8
Cystoscopy at 3 months					
positive v negative	12,16	881	143 (16)	2.2	1.8-2.7

Pooled univariable relative risks for tumour recurrence

Table 3.3b

Prognostic factor	Study references	Total patients	Patients with characteristic (%)	RR	95 % CI
Stage					
T1 v Ta	5,14,16,18	3311	1624 (49)	2.2	1.7-2.7
Grade					
2 v 1	5,14,16,18	2608	1724 (66)	2.2	1.5-3.1
3 v 1	5,14,16,18	1545	661 (43)	5.7	4.0-8.1
2-3 v 1	5,14,16,18	3269	2385 (73)	3.1	2.3-4.3
3 v 1/2	5,14,16,18	3269	661 (20)	3.6	2.9-4.4
Multiplicity					
multiple v solitary	14,18	2488	787 (32)	2.0	1.6-2.5
CIS presence					
CIS v without CIS	14,18	2393	355 (15)	4.4	3.4-5.5
Size (cm)					
>3 v <3	16,18	1622	294 (18)	1.7	1.3-2.3
Age					
>60 v <60	14,16	1577	1155 (73)	1.8	1.2-2.6
Gender					
female v male	14,16	1577	281 (18)	1.1	0.7-1.6

Pooled univariable relative risks for tumour progression

Table 3.3c

Prognostic factor	Study References	Total patients	Patients characteristic (%)	RR	95 % CI
Stage					
T1 v Ta	16,19-21	1547	775 (50)	1.0	0.8-1.2
Grade					
2 v 1	16,19,20	1013	443 (44)	2.0	1.4-2.7
3 v 1	16,19,20	711	141 (20)	3.3	2.2-4.9
2-3 v 1	16,19,20	1154	584 (51)	2.2	1.7-3.0
3 v 1/2	16,19-21	1500	214 (14)	2.5	1.9-3.3
CIS presence					
CIS v without CIS	19,21	707	37 (5)	4.0	2.4-6.5
Size (cm)					
>3 v <3	16,21	928	179 (19)	1.7	1.2-2.3
Age					
>60 v <60	16,20	845	610 (72)	3.5	2.2-5.5
Multiplicity					
multiple v solitary	19,21	699	218 (31)	1.7	1.2-2.5
Shape					
other v papillary	20-21	375	40 (11)	3.9	2.4-6.1

Pooled univariable relative risks for overall mortality

Tumour progression

Table 3.1 shows the prognostic factors considered by the four studies. Tumour grade and stage were considered in four studies[5, 14, 16, 18] and were found significant in four[5, 14, 16, 18] and one[5], respectively. The size of the tumour[5, 14, 16, 18] and the presence of carcinoma in situ (CIS)[5, 14, 16, 18] were studied in three studies and were found significant in all three.

Pooling showed that grade of disease (grade 3 compared to grade 1, RR 6.7; 95% CI 4.7-9.5) and the presence of CIS (RR 4.4; 95% CI 3.4-5.5, Table 3.3b) were the strongest prognostic factors for tumour progression. Grade 3 disease was present in 43% of the patients and CIS was present in 15% of the patients. Prognostic factors that had an intermediate effect were tumour stage (RR 2.2; 95% CI 1.7-2.7), multiplicity (RR, 2.0; 95% CI 1.6-2.5), age of first diagnosis (with 60 years cutoff, RR 1.8; 95% CI 1.2-2.6) and tumour size (with 3 cm cutoff, RR 1.7; 95% CI 1.3-2.3). Note that the prognostic factors for tumour progression were each stronger compared to the prognostic factors for tumour recurrence.

Mortality

In all four studies[16, 19-21] tumour grade and tumour stage were considered as prognostic factors for overall mortality and found significant in three[19-21] and one[21] respectively. Tumour size was considered in two studies significant in both[16, 21] (Table 3.1).

The strongest prognostic factors were the presence of CIS (pooled RR 4.0; 95% CI 2.4-6.5, Table 3.3c), a non-papillary shape of the tumour (RR 3.9; 95% CI 2.4-6.1), the age of the patient (with a cutoff of 60 years, RR 3.5; 95% CI 2.2-5.5) and grade 3 compared to grade 1 disease (RR 3.3; 95% CI 2.2-4.9). However only 5% of the studied patients had CIS and only 11% had a non-papillary tumour. Prognostic factors with an intermediate effect were tumour size (with 3 cm cutoff, RR 1.7; 95% CI 1.2-2.3) and multiplicity (RR 1.7; 95% CI 1.2-2.5). Tumour stage (RR 1.0; 95% CI 0.8-1.2) did not have a prognostic value for overall mortality.

Discussion

In this study we evaluated conventional clinical and pathological prognostic factors in bladder cancer for the endpoints recurrence, progression and overall mortality. We systematically combined evidence on classical clinical and pathological characteristics in NMI bladder cancer. The strongest factor to predict tumour recurrence was the result of the first 3-monthly cystoscopy after transurethral resection. For both progression and overall mortality we found that the differentiation grade of the tumour and the presence of CIS had the strongest effects.

Prediction of recurrence or the time to first recurrence is essential to determine the optimal interval for clinical follow up in the surveillance of urothelial cell carcinoma (UC). The result of the first 3-monthly cystoscopy had the strongest prognostic effect on recurrence (RR 2.2), yet

it has been considered in only a few studies[12, 16]. In contrast, multiplicity has been studied by many[5, 10, 12, 28, 29], nonetheless its pooled effect was rather modest (RR 1.4). Other prognostic factors, such as disease status and location of tumour, had stronger prognostic effects than multiplicity. Considering all prognostic factors for tumour recurrence, we only found weak to intermediate effects. We conclude that it is not possible to reliably predict time to recurrence with the current characteristics, let alone discriminate different patient groups at risk for recurrence.

Prediction of tumour progression may influence the policy on follow-up and treatment. High-grade tumours[5, 11, 28] and the presence of CIS[14, 18] were confirmed to be very strong prognostic factors. Moreover, except for patient gender, all the pooled prognostic factors showed intermediate to strong effects. So we confirm that we can predict which patients are at higher risk for tumour progression with clinical and pathological characteristics. This implies that the prognostic factors for progression can divide the UC population into different risk-groups, independent from the recurrence rate per year. As risk for progression is the major drive for intense surveillance it may even be conceivable that patients at low risk for progression would require less frequent follow-up irrespective of the predicted recurrence rate.

We also found relatively strong prognostic factors for overall mortality in NMI bladder cancer patients. The strongest prognostic factor was the presence of CIS. The strong prognostic effect of patient age[16, 20] and grade of tumour for overall mortality[19, 21, 30] were both confirmed in our meta-analysis. The shape of the tumour was also an important prognostic factor. However, the total numbers of patients studied for tumour shape were relatively small. Although stage is considered to be an important characteristic in survival studies for patients with NMI UC bladder cancer[5, 28, 30-32], we could not confirm this for the overall mortality risk (pT1 vs pTa: RR 1.0). Surprisingly, the mortality risk is precisely equal for patients with pTa or pT1 tumours. This may be explained in part by the heterogeneity of pT1 UC as is apparent from molecular and histopathological data. Recognition of this heterogeneity led pathologists to attempt substaging of pT1 UC in those with a NMI UC and with more invasive pattern[33].

Our meta-analysis has several strong points. First of all, a relatively large numbers of patients were assessed. This increased the power of the study as reflected in confidence intervals that are much smaller than in each of the studies. Second, the analysis included data of studies that showed no clear prognostic effect, thereby increasing the objectiveness of our findings. This is in contrast to classical reviewing, where the selection of studies may be biased. Furthermore three different endpoints were assessed for the most important clinical and pathological prognostic factors. Thus, the overall prognostic value of the various factors could be assessed more clearly.

However, like most meta-analyses, our study also has several limitations. Because of strict inclusion criteria we were only able to include eleven studies for tumour recurrence, four studies for tumour progression, and four studies for overall mortality. This reflects that the

latter two endpoints have not been studied as extensively in the literature. Another problem was that several definitions were used for the prognostic factors. Therefore, not all the available data could be analyzed. Other limitations were the differences in study designs, study population follow up policies, and the mixture of primary and/or recurrent tumours in patients. Finally, the treatment among the studied patients varied, which may have affected the effect of a prognostic factor, as in any prognostic study.

Kiemeney et al found that predictability in individual patients is highly inaccurate, but that the available prognostic factors in NMI bladder cancer may be useful to identify high and low risk subgroups[34]. Therefore, they concluded that more relevant prognostic factors are needed to decrease current over-treatment and under-treatment rates. Similarly, Witjes et[23] al concluded in a qualitative review that no single prognostic factor is able to predict prognosis in an individual patient with NMI UC bladder cancer. Thus a combination of multiple prognostic factors is needed to optimize current follow up policies.

This combination could consist of some of the clinical and pathological prognostic factors that are studied in the present meta-analysis together with prognostic factors based on molecular characteristics. Considerable information is already available regarding molecular pathogenesis and genetic prognostic factors of bladder cancer and is still evolving. Many oncogenes, gene products and tumour suppressor genes are identified (i.e. FGFR3, p53, p16, Rb). Especially the p53 and Rb genes are associated with progressive disease. Recently it has been demonstrated that FGFR3 mutations are especially prevalent in the NMI UC pTa group. In a retrospective study we showed that these mutations are associated with a low risk for progression. We also showed that molecular grading of bladder tumours using a combination of FGFR3 mutation status and high or low staining of the proliferation marker MIB-1 was superior in predicting progression than a combination of clinicopathological variables[35]. Moreover, it has been demonstrated that two mutually exclusive molecular pathways of bladder carcinogenesis can be identified, which correspond to UC with either a favourable or an aggressive clinical behavior[36]. Recent research shows that FGFR3 mutations are associated with the favourable pathway, whilst p53 mutations are found in the tumours with an unfavourable prognosis[37, 38]. Further research, preferably in a prospective setting, into the prognostic value of these markers and combinations of markers and factors is important to bring the ideal of individualized follow-up closer.

In conclusion, clinical and pathological prognostic factors had at most intermediate effects for tumour recurrence. There were a few strong prognostic factors for tumour progression and overall mortality. To enable individualization of surveillance strategies, current (clinical and pathological) prognostic factors need to be combined with markers found by researchers in the molecular field.

Table 3.4

Appendix: Estimation of relative risks (RR) and standard errors (SE) for tumor recurrence (rec), progression (pro) and overall mortality (mor). Not available (na).

Tumor stage (T1 v Ta)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Heney (5)	1.4	0.18	7.2	0.47	na	na
Smith (11)	1.0	0.35	na	na	na	na
Parmar (12)	1.3	0.23	na	na	na	na
Shinka (13)	1.4	0.26	na	na	na	na
Kiemeney (14)	1.4	0.09	2.4	0.20	na	na
Mulders (15)	1.3	0.17	na	na	na	na
Kurth (16)	1.0	0.11	2.0	0.24	1.1	0.18
Allard (8)	2.0	0.17	na	na	na	na
Kondo (17)	2.3	0.65	na	na	na	na
Millan-Rodriguez (18)	1.0	0.06	1.8	0.16	na	na
Flamm (19)	na	na	na	na	1.5	0.32
Lipponen (20)	na	na	na	na	2.0	0.41
Zieger (21)	na	na	na	na	0.8	0.12

Tumor shape (other v papilary)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Lipponen (20)	na	na	na	na	6.0	0.31
Zieger (21)	na	na	na	na	2.2	0.35

Multiplicity (multiple v solitary)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Dalesio (10)	2.1	0.14	na	na	na	na
Heney (5)	1.3	0.18	na	na	na	na
Parmar (12)	1.9	0.21	na	na	na	na
Shinka (13)	1.8	0.28	na	na	na	na
Kiemeney (14)	1.3	0.09	2.4	0.20	na	na
Mulders (15)	1.7	0.17	na	na	na	na
Allard (8)	2.1	0.14	na	na	na	na
Kondo (17)	2.7	0.61	na	na	na	na
Millan-Rodriguez (18)	1.3	0.06	1.9	0.14	na	na
Flamm (19)	na	na	na	na	2.2	0.32
Zieger (21)	na	na	na	na	1.5	0.24

Tumor grade (2 v 1)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Heney (5)	1.2	0.19	5.2	0.78	na	na
Smith (11)	1.2	0.37	na	na	na	na
Parmar (12)	1.1	0.21	na	na	na	na
Shinka (13)	1.3	0.29	na	na	na	na
Kiemeney (14)	1.2	0.10	2.2	0.30	na	na
Kurth (16)	1.3	0.13	2.9	0.31	1.6	0.21
Allard (8)	1.8	0.17	1.3	0.32	na	na
Kondo (17)	1.4	1.08	na	na	na	na
Millan-Rodriguez (18)	1.2	0.10	na	na	na	na
Flamm (19)	na	na	na	na	3.1	0.41
Lipponen (20)	na	na	na	na	2.5	0.33

Tumor grade (3 v 1/2)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Heney (5)	1.6	0.22	7.0	0.40	na	na
Smith (11)	1.1	0.40	na	na	na	na
Parmar (12)	1.9	0.26	na	na	na	na
Shinka (13)	1.4	0.31	na	na	na	na
Kiemeney (14)	1.1	0.11	3.8	0.20	na	na
Mulders (15)	1.1	0.20	na	na	na	na
Kurth (16)	1.1	0.18	2.6	0.28	1.2	0.29
Kondo (17)	2.6	0.63	na	na	na	na
Millan-Rodriguez (18)	1.0	0.07	3.4	0.15	na	na
Flamm (19)	na	na	na	na	2.6	0.33
Lipponen (20)	na	na	na	na	4.2	0.29
Zieger (21)	na	na	na	na	2.8	0.20

Tumor grade (2/3 v 1)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Dalesio (10)	1.4	0.16	9.6	0.74	na	na
Heney (5)	1.3	0.18	na	na	na	na
Smith (11)	1.2	0.33	na	na	na	na
Parmar (12)	1.3	0.18	na	na	na	na
Shinka (13)	1.4	0.26	na	na	na	na
Kiemeney (14)	1.2	0.09	3.4	0.28	na	na
Kurth (16)	1.3	0.12	3.3	0.30	1.6	0.20
Allard (8)	1.9	0.16	na	na	na	na
Kondo (17)	1.8	1.05	na	na	na	na
Millan-Rodriguez (18)	1.2	0.10	2.2	0.31	na	na
Flamm (19)	na	na	na	na	3.6	0.38
Lipponen (20)	na	na	na	na	3.4	0.30

Tumor grade (3 v 1)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Heney (5)	1.7	0.23	20.8	0.76	na	na
Smith (11)	1.2	0.43	na	na	na	na
Parmar (12)	1.9	0.27	na	na	na	na
Shinka (13)	1.5	0.34	na	na	na	na
Kiemeney (14)	1.2	0.13	6.4	0.30	na	na
Kurth (16)	1.3	0.20	5.1	0.36	1.6	0.31
Allard (8)	2.6	0.23	na	na	na	na
Kondo (17)	3.5	1.12	na	na	na	na
Millan-Rodriguez (18)	1.2	0.11	4.4	0.32	na	na
Flamm (19)	na	na	na	na	4.7	0.43
Lipponen (20)	na	na	na	na	6.5	0.35

Gender (female v male)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Kiemeney (14)	0.9	0.12	1.3	0.24	na	na
Mulders (15)	1.3	0.20	na	na	na	na
Kurth (16)	1.0	0.15	0.7	0.36	na	na
Allard (8)	1.0	0.16	na	na	na	na
Kondo (17)	0.9	0.78	na	na	na	na

Age (>60 v <60)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Kiemeney (14)	1.0	0.10	2.0	0.27	na	na
Kurth (16)	0.9	0.13	1.6	0.30	3.5	0.30
Lipponen (20)	na	na	na	na	3.5	0.35

Age (>65 v <65)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Mulders (15)	0.8	0.17	na	na	na	na
Kondo (17)	1.6	0.61	na	na	na	na

Cystoscopy at 3 months (positive v negative)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Parmar (12)	2.1	0.20	na	na	na	na
Kurth (16)	2.3	0.12	na	na	na	na

Disease status (recurrent v primary)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Dalesio (10)	1.9	0.14	na	na	na	na
Mulders (15)	1.2	0.19	na	na	na	na
Kurth (16)	1.4	0.12	na	na	na	na

Posterior wall involvement (yes v no)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Parmar (12)	1.4	0.20	na	na	na	na
Mulders (15)	2.0	0.20	na	na	na	na
Kurth (16)	1.3	0.11	na	na	na	na
Allard (8)	1.0	0.15	na	na	na	na

Trigone involvement (yes v no)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Mulders (15)	2.0	0.20	na	na	na	na
Kurth (16)	1.3	0.15	na	na	na	na
Allard (8)	1.2	0.29	na	na	na	na

CIS presence (yes v no)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Smith (11)	2.9	0.75	na	na	na	na
Shinka (13)	1.2	0.43	na	na	na	na
Kiemeney (14)	1.1	0.16	4.4	0.25	na	na
Allard (8)	1.6	0.59	na	na	na	na
Millan-Rodriguez (18)	1.0	0.08	4.3	0.14	na	na
Flamm (19)	na	na	na	na	8.6	0.32
Zieger (21)	na	na	na	na	1.1	0.41

Dysplasia presence (yes v no)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Heney (5)	1.3	0.21	na	na	na	na
Smith (11)	2.2	0.34	na	na	na	na
Allard (8)	0.8	0.37	na	na	na	na
Kiemeney (14)	1.1	0.12	na	na	na	na

Tumor size (>3 cm v <3 cm)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Dalesio (10)	1.2	0.15	na	na	na	na
Parmar (12)	1.8	0.19	na	na	na	na
Kurth (16)	1.0	0.15	1.9	0.26	1.4	0.22
Allard (8)	1.9	0.14	na	na	na	na
Millan-Rodriguez (18)	1.1	0.09	1.6	0.18	na	na
Zieger (21)	na	na	na	na	2.0	0.23

Tumor size (>2 cm v <2 cm)

First author (ref)	RR rec	SE rec	RR pro	SE pro	RR mor	SE mor
Mulders (15)	1.2	0.18	na	na	na	na
Kondo (17)	2.7	0.61	na	na	na	na

References

1. Greenlee RT, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. CA Cancer J Clin 2000;50:7-33.
2. Meijden AVD. Bladder Cancer. Bmj 1998;317:1366-9.
3. Kurth KH, Bouffioux C, Sylvester R, Van Der Meijden AP, Oosterlinck W, Brausi M. Treatment of superficial bladder tumours: achievements and needs. The EORTC Genitourinary Group. Eur Urol 2000;37 Suppl 3:1-9.
4. Millan-Rodriguez F, Chechile-Toniolo G, Salvador-Bayarri J, Palou J, Algaba F, Vicente-Rodriguez J. Primary superficial bladder cancer risk groups according to progression, mortality and recurrence. J Urol 2000;164:680-4.
5. Heney NM, Ahmed S, Flanagan MJ, Frable W, Corder MP, Hafermann MD, et al. Superficial bladder cancer: progression and recurrence. J Urol 1983;130:1083-6.
6. Rubben H, Lutzeyer W, Fischer N, Deutz F, Lagrange W, Giani G. Natural history and treatment of low and high risk superficial bladder tumours. J Urol 1988;139:283-5.
7. Herr HW. Intravesical BCG: current results, natural history and implications for urothelial cancer prevention. J Cell Biochem Suppl 1992;16I:112-9.
8. Allard P, Bernard P, Fradet Y, Tetu B. The early clinical course of primary Ta and T1 bladder cancer: a proposed prognostic index. Br J Urol 1998;81:692-8.
9. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. Pharmacoeconomics 2003;21:1315-30.
10. Dalesio O, Schulman CC, Sylvester R, De Pauw M, Robinson M, Denis L, et al. Prognostic factors in superficial bladder tumours. A study of the European Organization for Research on Treatment of Cancer: Genitourinary Tract Cancer Cooperative Group. J Urol 1983;129:730-3.
11. Smith G, Elton RA, Beynon LL, Newsam JE, Chisholm GD, Hargreave TB. Prognostic significance of biopsy results of normal-looking mucosa in cases of superficial bladder cancer. Br J Urol 1983;55: 665-9.
12. Parmar MK, Freedman LS, Hargreave TB, Tolley DA. Prognostic factors for recurrence and followup policies in the treatment of superficial bladder cancer: report from the British Medical Research Council Subgroup on Superficial Bladder Cancer (Urological Cancer Working Party). J Urol 1989; 142:284-8.
13. Shinka T, Hirano A, Uekado Y, Ohkawa T. Clinical study of prognostic factors of superficial bladder cancer treated with intravesical bacillus Calmette-Guerin. Br J Urol 1990;66:35-9.
14. Kiemeney LA, Witjes JA, Heijbroek RP, Debruyne FM, Verbeek AL. Dysplasia in normal-looking urothelium increases the risk of tumour progression in primary superficial bladder cancer. Eur J Cancer 1994;30A:1621-5.
15. Mulders PF, Meyden AP, Doesburg WH, Oosterhof GO, Debruyne FM. Prognostic factors in pTa-pT1 superficial bladder tumours treated with intravesical instillations. The Dutch South-Eastern Urological Collaborative Group. Br J Urol 1994;73:403-8.
16. Kurth KH, Denis L, Bouffioux C, Sylvester R, Debruyne FM, Pavone-Macaluso M, et al. Factors affecting recurrence and progression in superficial bladder tumours. Eur J Cancer 1995;31A:1840-6.
17. Kondo T, Onitsuka S, Ryoji O, Kihara T, Goto Y, Satoh T, et al. Analysis of prognostic factors related to primary superficial bladder cancer tumour recurrence in prophylactic intravesical epirubicin therapy. Int J Urol 1999;6:178-83.
18. Millan-Rodriguez F, Chechile-Toniolo G, Salvador-Bayarri J, Palou J, Vicente-Rodriguez J. Multivariate analysis of the prognostic factors of primary superficial bladder cancer. J Urol 2000;163:73-8.
19. Flamm J, Havelec L. Factors affecting survival in primary superficial bladder cancer. Eur Urol 1990; 17:113-8.
20. Lipponen PK, Eskelinen M, Jauhiainen K, Terho R, Harju E. Clinical prognostic factors in transitional cell cancer of the bladder. Urol Int 1993;50:192-7.
21. Zieger K, Wolf H, Olsen PR, Hojgaard K. Long-term survival of patients with bladder tumours: the significance of risk factors. Br J Urol 1998;82:667-72.
22. Aso Y, Anderson L, Soloway M, Bouffioux C, Chisholm G, Debruyne F, et al. Prognostic factors in superficial bladder cancer. Prog Clin Biol Res 1986;221:257-69.

23. Witjes JA, Kiemeney LA, Oosterhof GO, Debruyne FM. Prognostic factors in superficial bladder cancer. A review. Eur Urol 1992;21:89-97.
24. Glasziou P, Irwing L, Bain C. Systematic reviews in health care. Cambridge, United Kingdom: Cambridge University Press, 2001.
25. Harmer MH, International union against cancer. TNM classification of malignant tumours, ed. 3rd. Geneva: International Union against Cancer, 1978.
26. Mostofi F. International histological classification of tumours; in histological typing of urinary bladder tumours. Geneva: WHO, 1973.
27. Pettiti D. Statistical methods in meta-analysis, ed. 2nd. New York, NY: Oxford University Press, 2000.
28. Lutzeyer W, Rubben H, Dahm H. Prognostic parameters in superficial bladder cancer: an analysis of 315 cases. J Urol 1982;127:250-2.
29. Heney NM, Nocks BN, Daly JJ, Prout GR, Jr., Newall JB, Griffin PP, et al. Ta and T1 bladder cancer: location, recurrence and progression. Br J Urol 1982;54:152-7.
30. Takashi M, Murase T, Mizuno S, Hamajima N, Ohno Y. Multivariate evaluation of prognostic determinants in bladder cancer patients. Urol Int 1987;42:368-74.
31. Narayana AS, Loening SA, Slymen DJ, Culp DA. Bladder cancer: factors affecting survival. J Urol 1983;130:56-60.
32. Loening S, Narayana A, Yoder L, Slymen D, Penick G, Culp D. Analysis of bladder tumour recurrence in 178 patients. Urology 1980;16:137-41.
33. Platz CE, Cohen MB, Jones MP, Olson DB, Lynch CF. Is microstaging of early invasive cancer of the urinary bladder possible or useful? Mod Pathol 1996;9:1035-9.
34. Kiemeney LA, Witjes JA, Heijbroek RP, Verbeek AL, Debruyne FM. Predictability of recurrent and progressive disease in individual patients with primary superficial bladder cancer. J Urol 1993; 150:60-4.
35. Van Rhijn BW, Vis AN, Van Der Kwast Th, Kirkels WJ, Radvanyi F, Ooms EC, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. J Clin Oncol 2003;21:1912-21.
36. Spruck CH, 3rd, Ohneseit PF, Gonzalez-Zulueta M, Esrig D, Miyao N, Tsai YC, et al. Two molecular pathways to transitional cell carcinoma of the bladder. Cancer Res 1994;54:784-8.
37. Van Rhijn BW, Van Der Kwast Th, Vis AN, Kirkels WJ, Boeve ER, Jobsis AC, et al. FGFR3 and P53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. Cancer Res 2004;64:1911-4.
38. Bakkar AA, Wallerand H, Radvanyi F, Lahaye JB, Pissard S, Lecerf L, et al. FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder. Cancer Res 2003;63:8108-12.

CHAPTER 4

Prediction of progression of non-muscle invasive bladder cancer by WHO 1973 and 2004 grading and by *FGFR3* mutation status: a prospective study

Madelon NM van der Aa *
Maximiliaan Burger *
Johanna MM van Oers
A Brinkmann
Theo H van der Kwast
Ewout C Steyerberg
Ronald Stoehr
S Denzinger
Peter J Wild
WF Wieland
F Hofstaedter
Arnd Hartmann **
Ellen C Zwarthoff **

** Maximiliaan Burger and Madelon NM van der Aa contributed equally*

*** Arnd Hartmann and Ellen C Zwarthoff share senior authorship*

Abstract

The clinical management of non-muscle invasive urothelial cell carcinoma of the bladder (UC) is challenging, as it has a marked tendency to recur and to progress. Aim of the study was to investigate the prognostic value of the WHO 1973 and 2004 grading systems and biomarkers *FGFR3*, CK20 and Ki-67. In a prospective study, tumours from 221 patients were studied for the expression of CK20 and Ki-67 by immunohistochemistry and *FGFR3* status by SNaPshot mutation detection. Staging and grading were performed according to the WHO classification systems of 1973 and 2004. Median follow-up was 35 months. Recurrence occurred in 72/221 patients. None of the parameters was able to predict disease recurrence. CK20, Ki-67, *FGFR3* mutation, molecular grade using FGFR3 mutation analysis and Ki-67 and histological grading and staging were significantly associated with disease progression in stage. In multivariable analyses, both WHO 1973 and 2004 grading systems remained statistically significant and independent predictors of progression, with p=0.005 for WHO 1973 and p=.004 for 2004. *FGFR3* status was able to discriminate progressors from non-progressors in a subset of patients with high-grade UC (p=0.009).

This is the first prospective study comparing the WHO 1973 and 2004 grading systems. We show that both grading systems contribute valuable independent information. Therefore, it should be considered whether a yet better grading system could be developed that incorporates essential elements from both. The combination of WHO 2004 grading with *FGFR3* status allows a better risk stratification for patients with high-grade non-muscle invasive UC.

Introduction

Urothelial cell carcinoma of the bladder (UC) is a frequent neoplasm with an incidence of 30 cases per 100,000 in the western world.[1] At first presentation UC is mostly non-invasive or superficially invasive (i.e. pTa and pT1) and can be surgically controlled by transurethral resection. Up to 70% of patients have recurring disease requiring repeated surgery and about 10-15% of all cases will progress to muscle invasive UC.[2] To detect recurrent tumours, patients are followed by frequent cystoscopies. Bladder cancer is the second and tenth most prevalent cancer in males and females, respectively.[3] Indeed, it has been estimated that 1:1450 persons in the population are under surveillance for bladder cancer.[4] Consequently, the costs per patient of bladder cancer are the highest of all cancers.[3] Thus reliable prognostic parameters that can distinguish between high and low risk patients might enable clinicians to introduce individual risk adapted follow-up regimes.

To what extent a tumour is at risk for progression is mainly predicted by histological grade and stage, with higher risk attributed to higher grades. However histopathological classification is known to be limited by interobserver variability and may have varying prognostic implications.[5-7] In 1998, a revised grading system for UC was proposed and adopted by the World Health Organization (WHO) in 2004 to replace the WHO 1973 grading system[8], (Pathology and genetics of tumours of the urinary system an male genital organs, eds. JN Eble, G Sauter, JI Epstein and IA Sesterhenn: http://www.iarc.fr/WHO-BlueBooks/). Since then, the new system was discussed in a number of publications, however, its value is still a matter of debate.[9-13] Many pathologists still use the 1973 WHO classification and clinical decisions are based on the presence of grade 3 disease.

Interobserver variability in pathology has induced research into various molecular markers to improve the assessment of UC prognosis. Among the molecular markers are cytokeratin 20 (CK20) expression as marker of urothelial dedifferentiation, mutations in the gene for the fibroblast growth factor receptor 3 (*FGFR3*) and the proliferation marker Ki-67. Under normal conditions, CK20 is expressed in the superficial umbrella cells. CK20 expression in the entire urothelium or complete loss has been related to adverse outcome and disease recurrence in UC.[14,15] Activating mutations in the *FGFR3* gene were reported in up to 75% of low-grade and stage cases but are infrequent in muscle invasive UC.[7, 16-18] Several reports demonstrated that *FGFR3* mutations are associated with a favourable clinical course.[7,19,20] The proliferation index as determined by Ki-67 labelling has been linked to progression in non-muscle invasive UC.[21] The combination of *FGFR3* status and Ki-67 expression to a molecular grade was found to be a strong predictive factor in non-invasive and superficially invasive UC in a retrospective study. [7]

Aim of the present study was to evaluate the prognostic value of both WHO 1973 and 2004 grading systems and the molecular markers CK20, *FGFR3* and Ki-67 and molecular grade in a two-centred prospective setting. The results show that the 2004 grading system performs

slightly better than the WHO 1973 system in indicating progression in a univariate analysis, however a multivariable analysis shows that both grading systems contribute independent information. We also show that *FGFR3* status is able to differentiate between progressing and not progressing tumours in the group of high-grade carcinomas.

Material and Methods

Tumour samples

The study was approved by the appropriate Institutional Review Boards. Informed consent was obtained from all patients. 221 consecutive non-muscle invasive UC (pTa and pT1) obtained by transurethral resection at the Departments Urology of the University of Regensburg (n=111) and the Erasmus MC, Rotterdam (n=110) were investigated in a prospective study. The only selection criterion was the presence of sufficient tumour tissue after completion of the routine pathological diagnosis. Characteristics of the patients are given in table 4.1. Patients were followed according to appropriate guidelines[22] with a mean follow-up of 35 months (1–102 months). All slides were blindly reviewed by two uropathologists (A. H. and T. v. d. K.). Tumour stage and grade were assigned according to the WHO classification of malignant tumours of the urinary tract 2004 (Pathology and genetics of tumours of the urinary system an male genital organs, Eds. JN Eble, G Sauter, JI Epstein and IA Sesterhenn: http://www.iarc.fr/WHO-BlueBooks/). The initial grading data according to the 1973 WHO classification was reviewed by the same uropathologists.

Microdissection and DNA isolation

Genomic DNA was isolated from 5 µm paraffin sections of all tumours. Manual microdissection was performed to obtain at least 80% tumour cells. DNA was isolated using the QIAmp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's specifications. Elution with 2x100 µl of 70°C preheated water was performed to increase yield. Each elution step included a 5 min incubation of the QIAamp spin column with the preheated water at 70°C before centrifugation.

FGFR3 mutation analysis

FGFR3 mutation analysis was performed using the SNaPshot method.23 Three regions of the *FGFR3* gene, comprising all *FGFR3* mutations found in bladder cancer, were amplified simultaneously. After removal of excess primers and dNTPs, eight SNaPshot primers detecting nine *FGFR3* mutations (R248C, S249C, G372C, Y375C, A393E, K652E, K652Q, K652M, K652T) were annealed to the PCR products and extended with a labelled dideoxynucleotide. The extended primers were analyzed on an automatic sequencer.

Immunohistochemistry (IHC)

Immunohistochemical studies utilized an avidin-biotin peroxidase method with diaminobenzdine (DAB) chromatogen. After antigen retrieval (microwave oven for 30 min at 250W) IHC was carried out in a NEXES immunostainer (Ventana, Tucson, AZ, USA) following manufacturer's instructions. The following primary antibodies were used: anti CK20 (monoclonal, clone IT KS 20.8, Progen Biotech GmbH, Heidelberg, Germany; 1:100) and anti Ki-67 (rabbit monoclonal, clone MIB-1; Dianova, Hamburg, Germany; 1:800). Samples from Rotterdam were stained with anti Ki-67 (clone MIB-1, Conepta Biosystems, Barcelona, Spain; prediluted). Standard procedures were used for visualization (ABC-Elite, Vector-Laboratories, Burlingame, CA), and diaminobenzidine was used as chromogen. As negative controls all specimens were incubated without primary antibody. Immunoreactivity was scored according to validated criteria.[14,15,21] CK20 staining pattern was scored as normal if cytoplasmatic expression was seen in superficial umbrella cells only and as abnormal if a negative or >10% diffuse staining in the entire urothelium were noted. High Ki-67 labelling index was defined if ≥25% of the tumour cells showed nuclear immunoreactivity as previously described.[7]

Molecular Grade

Molecular grade (mG) was defined as a combination between *FGFR3* mutational status and Ki-67.[7,24] Molecular grade 1, mG1, is defined as *FGFR3* mutation and a low expression of Ki-67, mG2 as mutant *FGFR3* with high expression of Ki-67, or wild type *FGFR3* with a low expression of Ki-67 and mG3 as *FGFR3* wild-type in combination with high Ki-67 immunoreactivity.

Statistical analysis

Statistical analyses were completed using SPSS version 12.0 (SPSS, Chicago, IL). Two-sided Fisher's exact tests and Mann-Whitney-U-tests were used to study statistical associations between the various parameters. Progression was defined as progression in stage (i.e. pTa o pT1 or higher and pT1o pT2 or higher). Recurrence-free-survival (RFS), progression free survival (PFS) and overall survival (OS) curves comparing patients with the respective parameters were calculated using the Kaplan-Meier method, with significance evaluated by log rank test. Cox regression analysis was applied with a backward stepwise procedure, using $p<0.10$ for selection. All predictors for progression with $p<0.05$ for considered inclusion in the multivariable model. For the evaluation of RFS, patients were censored at the time of their last tumour-free clinical follow-up appointment or cystectomy. For OS analysis, patients were censored at the time of their last clinical follow-up appointment or at their date of death not related to the tumour. P values <0.05 were considered significant.

Results

Clinical data

Characteristics of the patients are summarized in table 4.1. Mean age at the time of diagnosis was 66.2 years (range 20 to 94). Gender distribution showed the expected male predominance with 171/221 male patients (77%). Only non-muscle invasive UC (pTa and pT1) were included in this study. In 152 (69%) cases UC were primary and in 69 (31%) recurring disease. Table 4.1 also shows the distribution of stage, grade and molecular markers for the selected tumours. Mean time of follow-up was 35 months (range 1-102 months). Out of 221 patients, 11 patients were lost to follow-up and 7 patients died of an unrelated disease. Recurrent disease was found in 72/221 (33%) patients; multiple recurrences were seen in 30/72 (41%) patients; two recurrences occurred in 18 (42%), three in 8 (17%) and four in 4 (7%) patients. Progression to higher stage was noted in 19/221 (9%) patients (11 patients with progression to ≥pT2 and 8 to pT1); 4 patients underwent cystectomy.

Table 4.1

Variable	Category	Patients	
		n	%
Age at diagnosis	≤ 68	120	50
	≥ 68	121	50
Gender	M	171	77
	F	50	23
Tumour stage	pTa	179	81
	pT1	42	19
WHO 1973 grade	G1	86	39
	G2	110	50
	G3	25	11
WHO 2004 grade	PUNLMP	49	22
	LG	119	55
	HG	50	23
Primary vs. recurrent	Primary	152	69
	Recurrent	69	31
FGFR3	wild type	80	36
	mutant	141	64
Ki-67 IHC	≤ 25%	146	72
	≥ 25%	57	28
CK20 IHC	normal	69	34
	abnormal	134	66
Molecular grade	mG1	106	52
	mG2	66	33
	mG3	31	15

Clinical, histopathological and molecular characteristics of the 221 patients

Relation of CK20, Ki-67 and FGFR3 and classical parameters

IHC for CK20 revealed abnormal staining pattern in 134/203 tumours (66%) cases. Ki-67 staining could be evaluated for 203 specimens and the proliferation index was high in 57 cases (28%). *FGFR3* mutation was observed in 141/221 (64%) of tumours (Table 4.1). The mutation most frequently detected was S249C (83/141; 59%), followed by Y375C (29; 21%) and R248C (19; 13%). Molecular Grade was defined in 203 specimens; 106 (52%) were categorized mG1, 65 (32%) mG2 and 31 (15%) as mG3, respectively (Table 4.1). Table 4.2 shows the distribution of molecular variables according to the WHO 1973 and 2004 grading systems. We found *FGFR3* mutational status to be related to normal CK20 expression and low Ki-67 labelling index as detected by IHC (Table 4.3).

Clinical outcome in relation to histopathological and molecular parameters

A Kaplan-Meier analysis for recurrence-free survival was performed on the 152 patients who presented with a primary tumour. However, statistical evaluation by the log rank test showed

Table 4.2

	FGFR3		Ki-67		CK20		Molecular Grade		
	mut	wt	low	high	nl	abnl	mG1	mG2	mG3
G1	65	21	68	6	42	36	55	17	2
G2	67	43	68	35	26	75	46	40	17
G3	9	16	9	16	1	23	5	8	12
p value	0.001		< 0.001		<0.001		< 0.001		
PUN-LMP	35	14	33	4	22	19	26	10	1
Low Grade	87	32	92	23	40	75	71	34	10
High Grade	16	34	18	29	5	39	7	20	20
p value	< 0.001		< 0.001		< 0.001		< 0.001		

WHO 1973 (G1, 2 and 3) and 2004 (PUNLMP, Low grade, High grade) grade related to molecular characteristics; P values by $\chi 2$ test, all numbers are absolute. Significant p values are in bold

Table 4.3

	FGFR3 mutation status		
Markers	Wild Type	Mutant	p value
CK20			
Normal pattern	15	54	
Abnormal pattern	54	81	0.009
Ki-67			
Low	40	106	
High	31	26	<0.001

Molecular Markers related to FGFR3 mutation status; P values by $\chi 2$ test, all numbers are absolute

that none of the variables measured was significantly related to the time to first recurrence although there was a trend towards a correlation between abnormal CK20 staining and recurrence (p=0.1).

Kaplan-Meier curves for progression-free survival are shown in fig. 1 and the data are summarized in table 4.4. WHO 1973 grade, WHO 2004 grade, pathological stage (pTa or pT1), CK20, *FGFR3,* Ki-67 status and molecular grade (mG) were significantly associated with progression in stage as determined by log rank tests (Table 4.4). The WHO 2004 grading system and

Table 4.4

	Progression			Log Rank
Prognostics	n	Progression	5 year progression free	p value [a]
CK20				
Normal pattern	63	2	92%	
Abnormal pattern	130	16	82%	0.05
Ki-67				
Low	137	9	91%	
High	54	9	72%	0.007
FGFR3				
Mutation	134	7	91%	
Wild type	76	12	74%	0.002
Molecular grade				
mG1	99	5	92%	
mG2	64	6	85%	
mG3	28	7	55%	0.0001
WHO Grade 1973				
G1	83	1	99%	
G2	107	12	79%	
G3	20	6	65%	0.003
WHO Grade 2004				
PUN-LMP	48	2	95%	
Low grade	114	5	91%	
High grade	45	12	62%	0.0000
Stage				
pTa	173	11	88%	
pT1	37	8	69%	0.004

Effect of Potential Prognostic Molecular Variables on Stage Progression in 221 Patients with UCC of the Bladder ([a]p value by log-rank test)

Molecular grade performed somewhat better in predicting progression than the WHO 1973 grading in univariate analysis (fig 1). In a subgroup analysis we observed that FGFR3 status was highly significant in predicting progression in the WHO high-grade tumours (p=0.009; fig.1, bottom right). Next we performed a multivariable analysis in which Cox regression analysis was applied with a backward stepwise procedure. All predictors for progression with p<0.05 were included in the multivariable model. After this stepwise procedure the variables WHO 1973 grade, WHO 2004 grade and *FGFR3* status remained. Both WHO 1973 and 2004 grading systems remained statistically significant and independent predictors of progression, with p=0.005 for WHO 1973 and p=0.004 for WHO 2004. Correlations between these classifications made the hazard ratios not interpretable. Presence of a FGFR3 mutation was associated with a lower risk of progression (hazard ratio=0.4, 95% confidence interval 0.1 - 1.2, p=0.095).

Discussion

The management of non-muscle invasive or "superficial" UC of the bladder is challenging for urologists and patients. Although the prognosis is generally good, up to 70% of patients show disease recurrence and up to 15% progression in grade or stage.[2] We describe here a prospective series of 221 non-invasive or superficially invasive UC from two urological centers (stage pTa or pT1). Recurrence was found in 72/221 (33%) and progression in 19/221 (9%) patients despite appropriate adjuvant therapy. The study population was followed for an average of 35 months. As in previous publications, mutant FGFR3, normal CK20 staining pattern and a low proliferation index (Ki-67 staining <25%) were predominant in well-differentiated tumours (G1/2 according to the 1973 WHO and PUNLMP/low-grade according to the 2004 WHO classifications). In addition, the presence of an *FGFR3* mutation was highly correlated to a normal CK20 expression pattern and lower proliferation rate as determined by Ki-67 (p=0.009 and p<0.001 respectively).

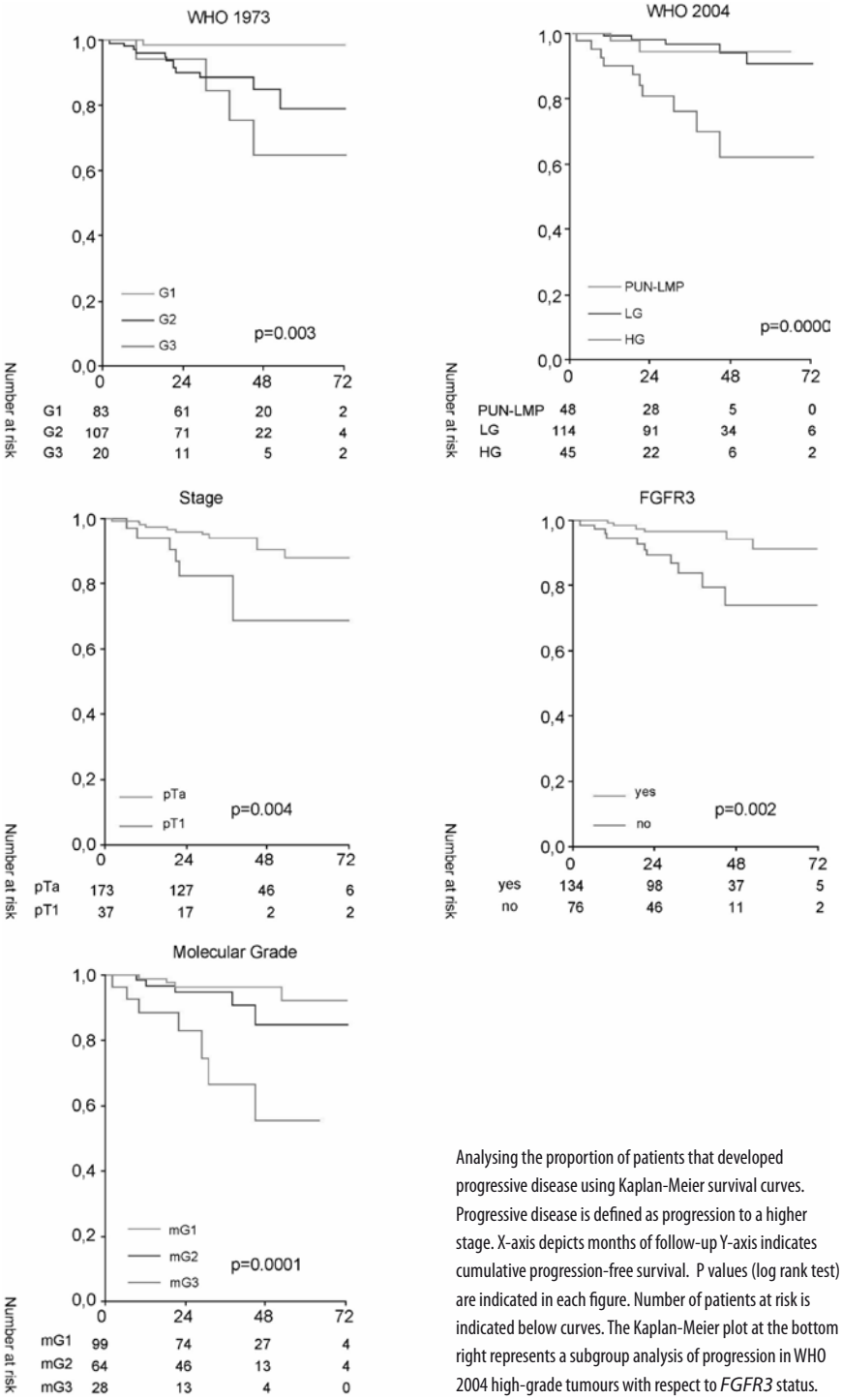
To date classical histopathological evaluation is the only method routinely used to determine the prognosis of the patients and to stratify therapy. Histopathology, however, is inherently subjective and displays inter- and intraobserver variability.[6,7] To improve reproducibility of diagnosis, a novel grading system featuring a specific category called papillary neoplasm of low malignant potential (PUN-LMP) and two grade categories was proposed in 1998 and adopted by the WHO in 2004,[8] (Pathology and genetics of tumours of the urinary system an male genital organs, eds. JN Eble, G Sauter, JI Epstein and IA Sesterhenn: http://www.iarc.fr/WHO-BlueBooks/). There is an ongoing debate about the added value of the WHO 2004 system versus that of 1973.[9,10,13,25-27] To our knowledge this is the first report that compares both grading systems on a prospectively sampled series of non-muscle invasive UC. We show that in the univariate analysis WHO 2004 grade and Molecular Grade can predict progression

more acccurately than WHO 1973 grade. However, in the multivariable analysis WHO grade 1973 and WHO grade 2004 apparently contribute independent valuable information that is relevant for prediction of progression in stage. In the WHO 1973 grading 12 of the progressing tumours were categorized as being grade 2 and 6 as being grade 3, whereas in the WHO 2004 system the high-grade tumours encompassed 12 of the 19 progressors (table 4.4). However, the WHO 2004 high-grade group is much larger (n=45) than the WHO 1973 grade 3 group (n=20). Samaratunga also compared both grading systems in relation to progression in a retrospective study. The WHO 2004 high-grade tumours in their study encompassed 50% of the progressing tumours (10 of 29), whereas the WHO 1973 G3 group comprised only 6 tumours of which 4 progressed.[28] It is our experience that additional information relevant for treatment decisions is provided to the clinicians by adding the distinction WHO grade 2 or 3 to the WHO 2004 high-grade tumours. Another matter of debate is the usefulness of the distinction between the PUNLMP and low-grade categories in the WHO 2004 grade.[10] In the Kaplan-Meier analysis of this prospective series of non-muscle invasive tumours we see no difference between the two groups with respect to prognosis prediction (figure 4.1, top right). In addition, the percentage of recurrences in both groups is equal (27%). Our data therefore support the view that the distinction between these two grades is not useful. In conclusion, according to the data of this prospective study we postulate the need for additional differentiation in the WHO 2004 high-grade category and we are not convinced that the distinction between PUNLMP and low-grade has additional value from a clinical point of view.

In order to enhance prognosis prediction, much effort is being spent on finding biomarkers that can be assayed in a reproducible manner and that have excellent predictive power. We have previously shown that *FGFR3* mutation analysis is 100% reproducible and an easy-to-perform multiplex assay for the 11 most frequent mutations has been developed (Van Oers, unpublished results). [7,23]Interestingly, in the WHO 2004 high-grade group, almost all FGFR3 wild type tumours (10 out of 11) progressed in the present study (figure 4.2). These results suggest that evaluation of FGFR3 mutation status enables the required distinction within the WHO high-grade group that we mentioned above. These results warrant a larger study into the added value of *FGFR3* mutations in UC of high-grade and raise the possibility that FGFR3 mutation analysis could define a high-grade non-muscle invasive tumour subgroup with very high progression risk. These patients could potentially benefit from an early aggressive therapy.

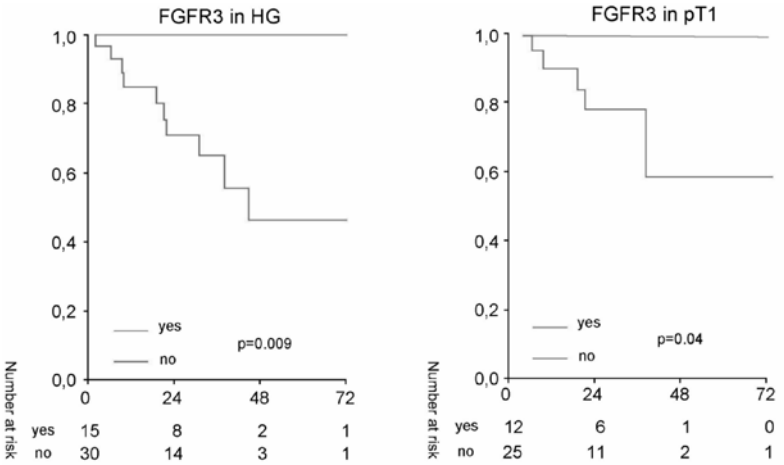
Ki-67 has been reported as a prognostic marker for disease and progression-free survival in pTa and pT1 UC.[21, 29] In the 203 pTa and pT1 tumours across all grades investigated in this study, we were able to confirm its relation to disease progression ($p<0.03$). The combination of Ki-67 with *FGFR3* mutational status, which has been defined as molecular grade before,[7]

Figure 4.1



Analysing the proportion of patients that developed progressive disease using Kaplan-Meier survival curves. Progressive disease is defined as progression to a higher stage. X-axis depicts months of follow-up Y-axis indicates cumulative progression-free survival. P values (log rank test) are indicated in each figure. Number of patients at risk is indicated below curves. The Kaplan-Meier plot at the bottom right represents a subgroup analysis of progression in WHO 2004 high-grade tumours with respect to *FGFR3* status.

Figure 4.2



Subgroup analyses using Kaplan-Meier survival curves for proportion of patients with progression to invasive disease within the WHO 2004 high-grade tumors (left) and tumors that were initially pT1 (right) in relation to FGFR3 status.
X-axis depicts months of follow-up; Y-axis indicates cumulative progression-free survival. p values (log-rank test) are indicated in each figure. Number of patients at risk is indicated below curves.

was associated with disease progression in our series and this combination can predict progression more accurately than either marker alone ($p<0.0001$) and was as efficient as the WHO 2004 grading system (figure 4.1) in the log rank test. When compared in the multivariable backwards COX regression analysis, molecular grade did not add significant information to both WHO grading systems and *FGFR3* status in this series of patients. However, it should be mentioned that the WHO 1973 and 2004 grades used in this work were based on a histopathology review by two expert urogenital pathologists. It is not unlikely that *FGFR3* status or molecular grading could significantly add to prognosis prediction in other settings because of their robustness and high reproducibility.

CK20 has been perceived as a prognostic factor for disease recurrence in non-invasive UC, [14, 15] but this has not been confirmed by others.[20,30] In the present study with 152 primary pTa and pT1 bladder tumours, we also could not confirm the prognostic power of abnormal staining for CK20 in relation to 5-year recurrence-free survival (p=0.1). None of the other parameters showed an association with recurrence in this series of tumours. Several reports have supported or did not support a relation between the occurrence of recurrence and *FGFR3*, Ki-67 and molecular grade.[20, 29, 31, 32] Recently, a on-line available decision model was proposed by Sylvester et al. based on a retrospective analysis of 2596 EORTC UC cases to predict the risk of recurrent disease in non-invasive UC . [33] Parameters in this model that in combination present different risk levels are multifocality, size, stage, presence of CIS, grade (WHO1973) and previous recurrences in case of non-primary tumours. Considering the contradictory outcomes of

the studies mentioned it is possible that the significance of molecular markers for recurrence risk can only be established from large studies as presented by Sylvester et al. These studies should include molecular markers like *FGFR3* status, CK20 expression pattern, Ki-67 labelling or gene expression profiles.

In conclusion, in this prospective study we showed that both the WHO 1973 and 2004 grading systems comprise independent valuable information for prediction of progression in stage in non-muscle invasive UC. There is a need for a further differentiation in the WHO 2004 high-grade group, either by making this distinction by adding WHO 1973 grade 2 and 3 or by adding *FGFR3* status, which, as we showed, enabled the identification of almost all progressors in the WHO 2004 high-grade non-muscle invasive UC.

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin. 2005 Mar-Apr;55(2):74-108.
2. Millan-Rodriguez F, Chechile-Toniolo G, Salvador-Bayarri J, Palou J, Vicente-Rodriguez J. Multivariate analysis of the prognostic factors of primary superficial bladder cancer. J Urol. 2000 Jan;163(1):73-8.
3. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. Pharmacoeconomics. 2003;21(18):1315-30.
4. Mariappan P, Smith G. A surveillance schedule for G1Ta bladder cancer allowing efficient use of check cystoscopy and safe discharge at 5 years based on a 25-year prospective database. J Urol. 2005 Apr;173(4):1108-11.
5. Witjes JA, Kiemeney LA, Schaafsma HE, Debruyn FM. The influence of review pathology on study outcome of a randomized multicentre superficial bladder cancer trial. Members of the Dutch South East Cooperative Urological Group. Br J Urol. 1994 Feb;73(2):172-6.
6. Tosoni I, Wagner U, Sauter G, Egloff M, Knonagel H, Alund G, et al. Clinical significance of interobserver differences in the staging and grading of superficial bladder cancer. BJU Int. 2000 Jan;85(1):48-53.
7. van Rhijn BW, Vis AN, van der Kwast Th, Kirkels WJ, Radvanyi F, Ooms EC, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. J Clin Oncol. 2003 May 15;21(10):1912-21.
8. Epstein JI, Amin MB, Reuter VR, Mostofi FK. The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. Am J Surg Pathol. 1998 Dec;22(12):1435-48.
9. Gonul, II, Poyraz A, Unsal C, Acar C, Alkibay T. Comparison of 1998 WHO/ISUP and 1973 WHO classifications for interobserver variability in grading of papillary urothelial neoplasms of the bladder. Pathological evaluation of 258 cases. Urol Int. 2007;78(4):338-44.
10. MacLennan GT, Kirkali Z, Cheng L. Histologic grading of noninvasive papillary urothelial neoplasms. Eur Urol. 2007 Apr;51(4):889-97; discussion 97-8.
11. Pavone-Macaluso M, Lopez-Beltran A, Aragona F, Bassi P, Fitzpatrick JM. The pathology of bladder cancer: An update on selected issues. BJU Int. 2006 Dec;98(6):1161-5.
12. Montironi R, Lopez-Beltran A. The 2004 WHO classification of bladder tumors: a summary and commentary. Int J Surg Pathol. 2005 Apr;13(2):143-53.
13. Epstein JI. The new World Health Organization/International Society of Urological Pathology (WHO/ISUP) classification for Ta, T1 bladder tumors: is it an improvement? Crit Rev Oncol Hematol. 2003 Aug;47(2):83-9.
14. Harnden P, Mahmood N, Southgate J. Expression of cytokeratin 20 redefines urothelial papillomas of the bladder. Lancet. 1999 Mar 20;353(9157):974-7.
15. Ramos D, Navarro S, Villamon R, Gil-Salom M, Llombart-Bosch A. Cytokeratin expression patterns in low-grade papillary urothelial neoplasms of the urinary bladder. Cancer. 2003 Apr 15;97(8):1876-83.
16. Cappellen D, De Oliveira C, Ricol D, de Medina S, Bourdin J, Sastre-Garau X, et al. Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet. 1999 Sep;23(1):18-20.
17. Billerey C, Chopin D, Aubriot-Lorton MH, Ricol D, Gil Diez de Medina S, Van Rhijn B, et al. Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am J Pathol. 2001 Jun;158(6):1955-9.
18. Knowles MA. Molecular subtypes of bladder cancer: Jekyll and Hyde or chalk and cheese? Carcinogenesis. 2006 Mar;27(3):361-73.
19. Hernandez S, Lopez-Knowles E, Lloreta J, Kogevinas M, Amoros A, Tardon A, et al. Prospective study of FGFR3 mutations as a prognostic factor in nonmuscle invasive urothelial bladder carcinomas. J Clin Oncol. 2006 Aug 1;24(22):3664-71.

20. van Oers JM, Wild PJ, Burger M, Denzinger S, Stoehr R, Rosskopf E, et al. FGFR3 Mutations and a Normal CK20 Staining Pattern Define Low-Grade Noninvasive Urothelial Bladder Tumours. Eur Urol. 2007 Jan 12.
21. Lopez-Beltran A, Luque RJ, Alvarez-Kindelan J, Quintero A, Merlo F, Requena MJ, et al. Prognostic factors in survival of patients with stage Ta and T1 bladder urothelial tumors: the role of G1-S modulators (p53, p21Waf1, p27Kip1, cyclin D1, and cyclin D3), proliferation index, and clinicopathologic parameters. Am J Clin Pathol. 2004 Sep;122(3):444-52.
22. Smith JA, Jr., Labasky RF, Cockett AT, Fracchia JA, Montie JE, Rowland RG. Bladder cancer clinical guidelines panel summary report on the management of nonmuscle invasive bladder cancer (stages Ta, T1 and TIS). The American Urological Association. J Urol. 1999 Nov;162(5):1697-701.
23. van Oers JM, Lurkin I, van Exsel AJ, Nijsen Y, van Rhijn BW, van der Aa MN, et al. A simple and fast method for the simultaneous detection of nine fibroblast growth factor receptor 3 mutations in bladder cancer and voided urine. Clin Cancer Res. 2005 Nov 1;11(21):7743-8.
24. van der Aa MN, van Leenders GJ, Steyerberg EW, van Rhijn BW, Jobsis AC, Zwarthoff EC, et al. A new system for substaging pT1 papillary bladder cancer: a prognostic evaluation. Hum Pathol. 2005 Sep;36(9):981-6.
25. Lopez-Beltran A, Montironi R. Non-invasive urothelial neoplasms: according to the most recent WHO classification. Eur Urol. 2004 Aug;46(2):170-6.
26. Yorukoglu K, Tuna B, Dikicioglu E, Duzcan E, Isisag A, Sen S, et al. Reproducibility of the 1998 World Health Organization/International Society of Urologic Pathology classification of papillary urothelial neoplasms of the urinary bladder. Virchows Arch. 2003 Dec;443(6):734-40.
27. Oosterhuis JW, Schapers RF, Janssen-Heijnen ML, Pauwels RP, Newling DW, ten Kate F. Histological grading of papillary urothelial carcinoma of the bladder: prognostic value of the 1998 WHO/ISUP classification system and comparison with conventional grading systems. J Clin Pathol. 2002 Dec;55(12):900-5.
28. Samaratunga H, Makarov DV, Epstein JI. Comparison of WHO/ISUP and WHO classification of noninvasive papillary urothelial neoplasms for risk of progression. Urology. 2002 Aug;60(2):315-9.
29. Quintero A, Alvarez-Kindelan J, Luque RJ, Gonzalez-Campora R, Requena MJ, Montironi R, et al. Ki-67 MIB1 labelling index and the prognosis of primary TaT1 urothelial cell carcinoma of the bladder. J Clin Pathol. 2006 Jan;59(1):83-8.
30. Alsheikh A, Mohamedali Z, Jones E, Masterson J, Gilks CB. Comparison of the WHO/ISUP classification and cytokeratin 20 expression in predicting the behavior of low-grade papillary urothelial tumors. World/Health Organization/Internattional Society of Urologic Pathology. Mod Pathol. 2001 Apr;14(4):267-72.
31. van Rhijn BW, Lurkin I, Radvanyi F, Kirkels WJ, van der Kwast Th, Zwarthoff EC. The fibroblast growth factor receptor 3 (FGFR3) mutation is a strong indicator of superficial bladder cancer with low recurrence rate. Cancer Res. 2001 Feb 15;61(4):1265-8.
32. van Rhijn BW, Lurkin I, Chopin DK, Kirkels WJ, Thiery JP, van der Kwast Th, et al. Combined microsatellite and FGFR3 mutation analysis enables a highly sensitive detection of urothelial cell carcinoma in voided urine. Clin Cancer Res. 2003 Jan;9(1):257-63.
33. Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol. 2006 Mar;49(3):466-5; discussion 75-7.

CHAPTER 5

A New System For Substaging pT1 Papillary Bladder Cancer: a Prognostic Evaluation

Madelon NM van der Aa
Geert JLH van Leenders
Ewout W Steyerberg
Bas W van Rhijn
Adriaan C Jöbsis
Ellen C Zwarthoff
Theodorus H van der Kwast

Abstract

Superficially invasive (pT1) papillary urothelial cell carcinomas (UC) may run a variable course. Several attempts have been made for substaging of UC in order to identify the clinically aggressive tumours. We present a new substaging system, based on the extent of invasion. From a series of 53 primary pT1 UC, 24 cases showed invasion of the subepithelial stroma by an invasive front extending over a maximum length of 0.5 mm (pT1mic) and 29 showed extensively (>0.5mm) infiltrating UC (pT1ext). We tested diagnostic reproducibility between two pathologists and found 81% agreement. Furthermore, all cases were analysed for mutations in the *fibroblast growth factor receptor 3 (FGFR3) gene*, which represents the favourable pathway of urothelial cell carcinogenesis. Mutant *FGFR3* was commonly observed in pT1mic UC (15/24, 63%), but rarely (2/29, 7%) in pT1ext UC (χ^2 – test, p-value <0.001). The presence of pT1ext at initial diagnosis proved to be the strongest predictor for progression, also when adjusted for FGFR3 mutation status in a Cox regression model. This relatively simple, new sub-staging system for pT1 UC may prove to be of prognostic value and supportive to clinical decision-making.

Introduction

The clinical course of non-muscle invasive (pTa and pT1) papillary urothelial cell carcinoma (UC) is characterized by a high tendency to recur (up to 70%) and a propensity to progress in grade (10-30%) or stage (10-15%).[1] The follow-up policy and treatment of patients with non-muscle invasiveUC predominantly depends on conventional parameters like grade and stage (pTa versus pT1) and the associated presence of flat lesions like carcinoma in situ. [2,3] For the subset of papillary non-muscle invasiveUC that invades into the sub-epithelial connective tissue (pT1), the clinical management is not standardized, since the biologic behavior of individual cases remains elusive.[4, 5] Besides, molecular data point at the heterogeneous nature of pT1 UC.[6] Clinicians are inclined to treat pT1-tumours, in particular the ones with high-grade in combination with carcinoma in situ (CIS), more aggressively, because their higher risk of progression to muscle invasive UC. [7, 8]

Various approaches have already been put forward to identify those with more aggressive behavior among pT1 UC. These include molecular characteristics such as over-expression of the p53 protein, proliferation markers like MIB-1 and DNA-ploidy status. [9, 10] However, because of the limited prognostic impact or for technical reasons these molecular markers have not been introduced in routine pathology of UC.[11]

The depth of tumour infiltration in the submucosa has also been put forward as an approach to perform substaging in pT1 UC. This could be done by direct measurement of the depth of invasion with a micrometer or by the assessment of invasion relative to the muscularis mucosa. [12, 13] These methods have not yet gained a wide acceptance. [14] There is no fixed standardized orientation of tissue-structures in transurethrale-resected tissue complicating the quantification of perpendicular tumour invasion and identification of histological landmarks as muscularis mucosae and vascular plexus. [15]

In this study, we set out to quantify the horizontal tumour invasion. This approach for subdividing papillary pT1 UC is based on the extent of the invasive front. By measuring the maximum length of extent, parallel to the overlying epithelium, it is possible to distinguish micro-invasion from "extensive" infiltration. This method may be less dependent on orientation of the tissue specimen and represents a practical definition that is generally applicable. We aimed assess the reproducibility of this new sub-staging system and its prognostic value.

Recently, two mutually exclusive molecular pathways of bladder carcinogenesis have been identified corresponding to UC with either a favorable or an aggressive clinical behavior. [6] Accordingly, p53 over-expression defines UC with high recurrence rate and increased likelihood of progression, as mutations in the gene encoding the fibroblast growth factor receptor 3 (*FGFR3*) are characteristic of UC with favorable prognosis. [10,15] Therefore, we also evaluated the correlation between sub-staging and *FGFR3* mutation status and assessed the prognostic effect of the substaging system while adjusting for *FGFR3* status.

Materials and Methods

Patients and follow-up

From a previously described cohort of 286 patients with papillary UC we extracted 63 formerly diagnosed pT1 UC tumours. [10] After central pathological review (ThvdK) of the pT1 tumours, 53 tumours remained to evaluate. For reason of comparison, the files of 131 patients with pTa UC were retrieved to obtain data on their follow-up. The patients were seen at the departments of Urology at the Erasmus Medical Center Rotterdam (n=33) and at the Sint Franciscus Gasthuis, Rotterdam (n=20). Mean age of patients at diagnosis was 68 ± 10 years (range 47 - 90y). All patients were diagnosed with UC for the first time. After first diagnosis 42 patients (79%) were treated with immuno- or chemotherapy by intra-vesicle instillation during median time of follow-up of 55 months (range 9-228 months). Ten patients (19%) died of disease, 8 patients (15%) underwent cystectomy and 28 progressed in disease. Of these 28, tumour recurred as muscle invasive (≥ pT2) in 16 (30%) patients, 9 progressed in grade (G3) with concomitant CIS and 3 were considered as clinically progressed because of repeated recurring pT1G3 + CIS which was treated by cystectomy.

A recurrence was counted if it occurred after at least 3 months after diagnosis. Recurrence rate was calculated as the total number of recurrences divided by the total number of months of follow-up times 12 months. Progression was defined as a histologically diagnosed recurrence with higher grade, stage or the appearance of carcinoma in situ. Also included in the definition of progression was cystectomy performed on patients with recurring pT1G3 associated with concomitant carcinoma in situ. Progression was the primary end-point in this study, since it is clinically the most relevant.

Histopathology and Immunohistochemistry

Standard haematoxylin and eosin (H&E) stained slides of formerly diagnosed pT1 UC were reviewed by a specialized genitourinary pathologist (THvdK) and for reasons of comparison graded, both according to the 1973 WHO classification for urothelial neoplasm and according to 1998 WHO/ISUP system. [16] Tumours were staged according to WHO 2002 TNM classification guidelines. [1] All 53 specimens showed invasion into the underlying lamina propria but no infiltration into the muscularis propria (pT1).

Next, two different types of tumour invasion were distinguished, i.e. focal or micro-invasive and extensive pattern of invasion (Figure 5.1). Micro-invasion was defined as a single focus of invasion of the subepithelial stroma by an invasive front, parallel to the overlying (neoplastic) urothelium over a maximum distance of 0.5 mm (within one high power field, objective X 40). Extensive infiltration could either be multifocal micro-invasive areas or invasion by tumour areas that would not fit within one high power field. All slides were independently reviewed by two specialized pathologists (THvdK&GvL) for the extent of infiltration into the stroma.

To assess proliferative capacity all samples were stained with a murine monoclonal antibody MIB-1 (Immunotech, Marseille, France) using antigen retrieval by microwave and visualization was achieved by an avidin-biotin peroxidase complex method as previously described. [17] Molecular grading is defined as combination of FGFR3 status with MIB-1 expression.[10] Pathological review of the slides was carried out without knowledge of the clinical or molecular status.

Molecular analysis for *FGFR3* mutation

The HE-slides served as templates for dissection to obtain DNA from tumour, avoiding contamination with stroma, normal mucosa. The samples used for *FGFR3* mutation analysis contained a minimum of 70% tumour cells, as assessed by histological examination. DNA was extracted using the DNeasy Tissue Kit (Qiagen GmbH, Hilden Germany). *FGFR3* mutation analysis was performed by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) analysis on tumour DNA and, if available, control DNA from venous blood samples of the patients as previously described. [18, 19, 20] Three regions of interest encompassing activating *FGFR3* mutations previously described in severe skeletal dysplasias and cancers were amplified by PCR. The primer sequences were as reported. The laboratory analyses were performed without knowledge of clinical or histopathological status. A detailed description of *FGFR3* mutation analysis was given previously. [10]

Statistics

Statistical analyses were performed using SPSS 11.0 software (SPSS Inc., Chicago, IL). The statistical significance of the correlation between pattern of invasion and clinical and pathological variables was assessed using Chi-square tests and Mann-Whitney tests. The cumulative incidences of progression were estimated by means of the Kaplan-Meier Method and compared using the log-rank test. Cox regression model was used to analyze combinations of the extent of infiltration and other prognostic factors. $P < 0.05$ was considered statistically significant.

Results

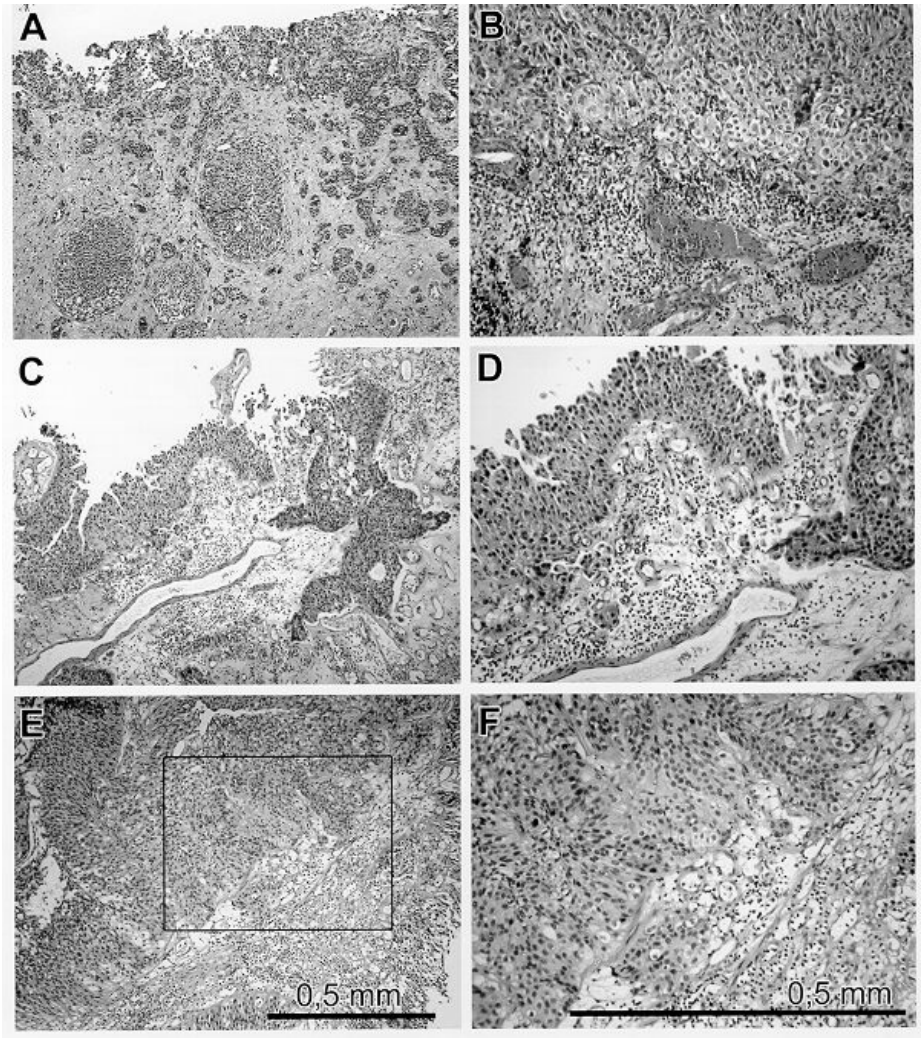
Twenty-four of the 53 pT1UC displayed focal invasion (pT1mic) and 29 were categorized as extensively invasive (pT1ext). The histopathological classification of non-muscle invasiveUC is subdued to a significant interobserver variability. In order to test the diagnostic reproducibility of tumour invasion as presented in this study, two pathologists revised all 53 primary tumours for stage and substage (table 5.1). In 43 (81% agreement) tumours both pathologists scored the same diagnosis regarding the extent of invasion. The tumour characteristics in relation to the two patterns of invasion in this series of 53 patients are listed in table 5.2. Grading (WHO '73 and WHO/ISUP '98), *FGFR3* mutation status, MIB-1 expression and molecular grading were significantly correlated with the extent of invasion.

Table 5.1

81% agreement	Pathologist 2			
Pathologist 1	pTa	PT1foc	PT1ext	Total
PT1 foc	8	14	2	24
PT1 ext	0	0	29	29
Total	8	14	31	53

Scores in agreement of substaging pT1 by two expert pathologists. Agreement (in %) in histological data of 53 patients formerly diagnosed with primary papillary pT1 UC scored by two expert urogenital pathologists for the new system for substaging

Figure 5.1



Photomicrographs illustrate pT1 UC with extensively infiltrating (A-D) and micro-invasive (E, F) patterns. In A and B, the invasive tumor fronts are not contained within 1 HPF, or multiple foci of invasion are present (C, D). Details are given at high power in B, D, and F (x200).

Table 5.2

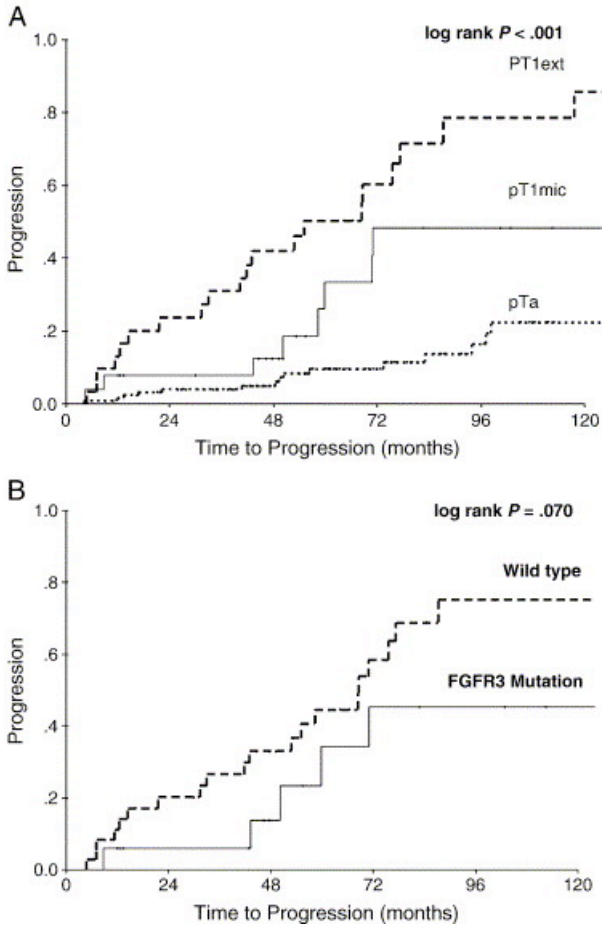
Variables	Micro-invasion (n=24)	Extensive Invasion (n=29)	P value [a]
Mean Age (years) ± SD	- 69 ± 10	- 67 ± 10	0.40
Gender			
- male	- 19	- 23	
- female	- 5	- 6	0.99
Grade WHO '73			
- G1	- 2 (8%)	- 0	
- G2	- 15 (63%)	- 9 (31%)	
- G3	- 7 (29%)	- 20 (69%)	.002
Grade WHO/ISUP '98			
- Low-grade	- 10 (42%)	- 4 (14%)	
- High-grade	- 14 (58%)	- 25 (86%)	.022
Molecular Status			
- FGFR3 mutation o	- 15 (63%)	- 2 (7%)	
- wild type	- 9 (37%)	- 27 (93%)	.000
IHC, MIB-1 Staining			
- MIB < 25%	- 12 (50%)	- 4 (14%)	
- MIB ≥ 25%	- 12 (50%)	- 25 (86%)	.004
Molecular Grading			
- mG1 (mt/≤ 25%)	- 6 (46%)	- .	
- mG2	- 3 (23%)	- 6 (21%)	
- mG3 (wt /≥25%)	- 4 (31%)	- 23 (79%)	.000
CiS at Diagnosis			
- yes	- 2 (8%)	- 5 (17%)	
- no	- 22 (92%)	- 24 (83%)	.340

Pathological and molecular characteristics related to the extent of infiltration, micro- and extensive invasion, of 53 patients with primary pT1 UCC of the bladder [a] P value by $\chi 2$ test

To evaluate the clinical importance of pT1 UC in these two subsets, its prognostic impact was compared with that of other potentially prognostic variables for progression as endpoint (Table 5.3). Micro-invasion was the most important predictor of progression. The Kaplan-Meier curves further illustrate the significant impact of pT1 sub-staging (Figure 5.2a) and the impact of FGFR3 gene mutation (Figure 5.2b) on progression. A highly significant difference in progression was noted between patients with an initial diagnosis of pT1mic and pT1ext UC.

Table 5.3 shows the hazard ratios of the different variables. Extended invasion has a three times higher risk of progression, HR=3.0 in univariate analysis. Taking into account the possible impact of other variables, we found that the risk of progression remained almost similar (multivariate, HR=2.7).

Figure 5.2



Kaplan-Meier Curves;
A Distribution of Time to Progression in Population of 53 pT1 UC and 131 pTa UC
B Distribution of Time to Progression for FGFR3 status in Population of 53 pT1 UC

Discussion

We describe a new system for substaging pT1 tumours that is valuable as prognostic factor and supportive in clinical decision-making. We found that scoring of the extent of invasion, using this method, is reasonably reproducible. There is a clear correlation with other prognostic factors, especially with FGFR3 mutation status. Furthermore, the extent of invasion was the most independent predictor for progression.

Invasive pT1 UC may be derived from carcinoma in situ of the urothelium or from papillary UC. It is well recognized that pT1 UC originating from carcinoma in situ (CIS) carries a substantial risk of progression to muscle invasive UC. For a subset of invasive UC accompanied by CIS, some authors employed the term "micro-invasive" UC. [4, 21, 22] The definition of micro-invasive carcinoma in the context of CIS in these studies was invasion over a front > 2mm or < 5 mm.

Table 5.3

Prognostics	n	Progression	5y progression free	P value [a]	Univariate HR [CI 95%] [b]	Multivariate HR [CI 95%] [b]
Micro-invasion						
- Micro-invasion o	24	9	69%		3.0	2.7
- Extensive invasion	29	19	50%	.009	[1.3-7.2]	[0.9-8.4]
Tumor Grade WHO '73						
- G1	2	0	100%		1.9	
- G2	24	12	50%		[0.5-2.0]	
- G3	27	16	64%	.85		
Tumor Grade WHO/ISUP '98						
- Low-grade	14	5	58%		0.97	
- High-Grade	39	23	59%	.95	[0.4-2.6]	
Molecular Status						
- FGFR3 mutation	17	7	66%		0.4	0.8
- wild type	36	21	55%	.07	[0.2-1.1]	[0.2-3.0]
IHC, MIB-1 Staining						
- MIB < 25%	16	7	66%		1.9	
- Mib ≥ 25%	37	21	55%	.18	[0.8-4.6]	
Molecular grading						
- mG1	10	4	72%			
- mG2	13	6	65%		1.8	
- mG3	30	18	52%	.14	[1.0-3.2]	
CiS at Diagnosis						
- yes	7	6	57%		0.9	
- no	46	22	59%	.87	[0.3-2.5]	

Effect of potential prognostic variables on clinical progression in 53 patients with primary pT1 UCC of the bladder. [a] P value by log-rank test [b] Hazard Ratio and 95% confidence interval by Cox regression analysis

Some authors considered this cut-off point of 5 mm too advanced, since about 6% of these patients had metastatic disease. [23, 13]

Despite a proposal in 1993 to distinguish micro-invasive from other pT1 UC, the terminology of micro-invasive carcinoma for papillary UC is not well established. [12] The latter study emphasized the heterogeneity of the pT1 tumour group and the problem in the assessment of micro-invasion. The authors hypothesized a difference in behavior but did not examine the biologic basis and clinical value of a sub-staging system. In a more recent study on TUR specimens, it was demonstrated that the depth of lamina propria invasion, as measured by a micrometer, proved to be predictive for progression. A highly significant difference in progression was noted between patients with UC with infiltration depth < 0.5 mm and > 1.5 mm. [24] This finding provides strong support to the concept that separation of micro-invasive papillary UC from those with a deeper infiltration is of real clinical importance.

A potential disadvantage of the measurement of the depth of invasion as a single criterion may be the lack of orientation of the tissue from a TUR specimen or bladder biopsy. To circumvent the lack of tissue orientation, similar approaches have been put forward, but based on the depth of infiltration of pT1 UC in relation to the vascular plexus in the submucosa.[13]

Unfortunately it has been shown to be difficult for the proposed sub-staging systems to gain a wide acceptance. This may be attributed to discrepant literature data on clinical relevance, inter-observer variation among pathologists and practical problems in the application of the sub-staging due to lack of orientation of the specimen. Orientation in biopsies and transurethral resection specimens as well as cauterization artifacts may indeed underlie the variability in interpretation by pathologists. A recent study, however, claimed to improve the accuracy of substaging pT1 UC into pT1a and pT1b with the aid of immunohistochemistry using antibodies to desmin and cytokeratin. [21]

Here, we report on a relatively simple, dichotomized categorization of papillary pT1 UC, separating those with focal or micro-invasion from pT1 UC with an extensive invasion pattern. By taking into account the maximum length of infiltration parallel to the urothelial surface, the problem of poor orientation is largely circumvented. Our definition of micro-invasive papillary pT1 UC on the basis of the length of the infiltrating tumour area confined within 1 hpf may be considered rather inaccurate, but its advantage is the relative simplicity, which may increase the compliance of the pathologist.

To test diagnostic reproducibility a second pathologist, instructed only by a manual, independently scored substage in all 53 pT1 tumours. Both pathologists found agreement in 81% of cases. As shown in table 5.1 there was disagreement in 8 cases, tumours diagnosed pT1mic by the first pathologist, were considered pTa (mimicking invasion into the submucosa, caused by tissue damage) by the second pathologist, the reviewer. Considering agreement between pathologists on histological features, this substaging system should be reasonably applicable. [14,25]

The observation of the frequent occurrence of the *FGFR3* mutation in pT1mic as compared to pT1ext UC can be considered as a strong argument that this simple method of pathologic sub-staging of pT1 UC is of biologic relevance. On the other hand, we note that FGFR3 mutation had no independent effect on progression when taking into account the extent of invasion. However, the extent of invasion proved to be the most important independent prognostic factor for progression free survival, clearly surpassing *FGFR3* mutation status.

Since progression is largely determined by the stage and grade of subsequent recurrent UC, it may seem curious that features in the pT1 UC, removed at diagnosis can serve as a prognosticator. Currently, evidence has been given that the majority of primary and recurrent UC have a monoclonal origin. [13, 24] This offers a good explanation for the phenomenon that primary and recurrent UC share a common biology, and that the pattern of invasion at diagnosis reflects a future pathway.

Although it would be advantageous to perform substaging of pT1 UC, the problem of understaging in TUR specimens should also be considered: one study reported that 25% of high-grade UC diagnosed on the TUR as pT1 revealed a pT2 or pT3 UC in the corresponding cystectomy specimen. [9] Thus, it maybe that a proportion of our pT1ext represent underdiagnosed pT2 UC.

In a previous study we demonstrated that the recurrence rate of UC was related to the *FGFR3* mutation status, i.e. those patients with (pTa and pT1) UC carrying the *FGFR3* mutation genotype showed less frequent recurrences as compared to those with the wild type FGFR3 genotype. [5, 27] In this series of patients with pT1 UC we could not confirm this observation. Time to first recurrence is considered an important parameter to determine the optimal interval for clinical follow-up in surveillance of UC. Mean time to first recurrence was 21 months (range 4 – 116 months). This series of pT1 UC included 16 patients who did not develop a recurrence. Thirteen of these patients were free of recurrence for a longer period of follow-up than the mean time to first recurrence in this study. We were not able to demonstrate a specific prognostic feature present in these 16 patients. It is likely that these UC had a good therapy response, leading to complete eradication of the UC after TUR in the absence of implantation of UC cells elsewhere in the bladder mucosa.

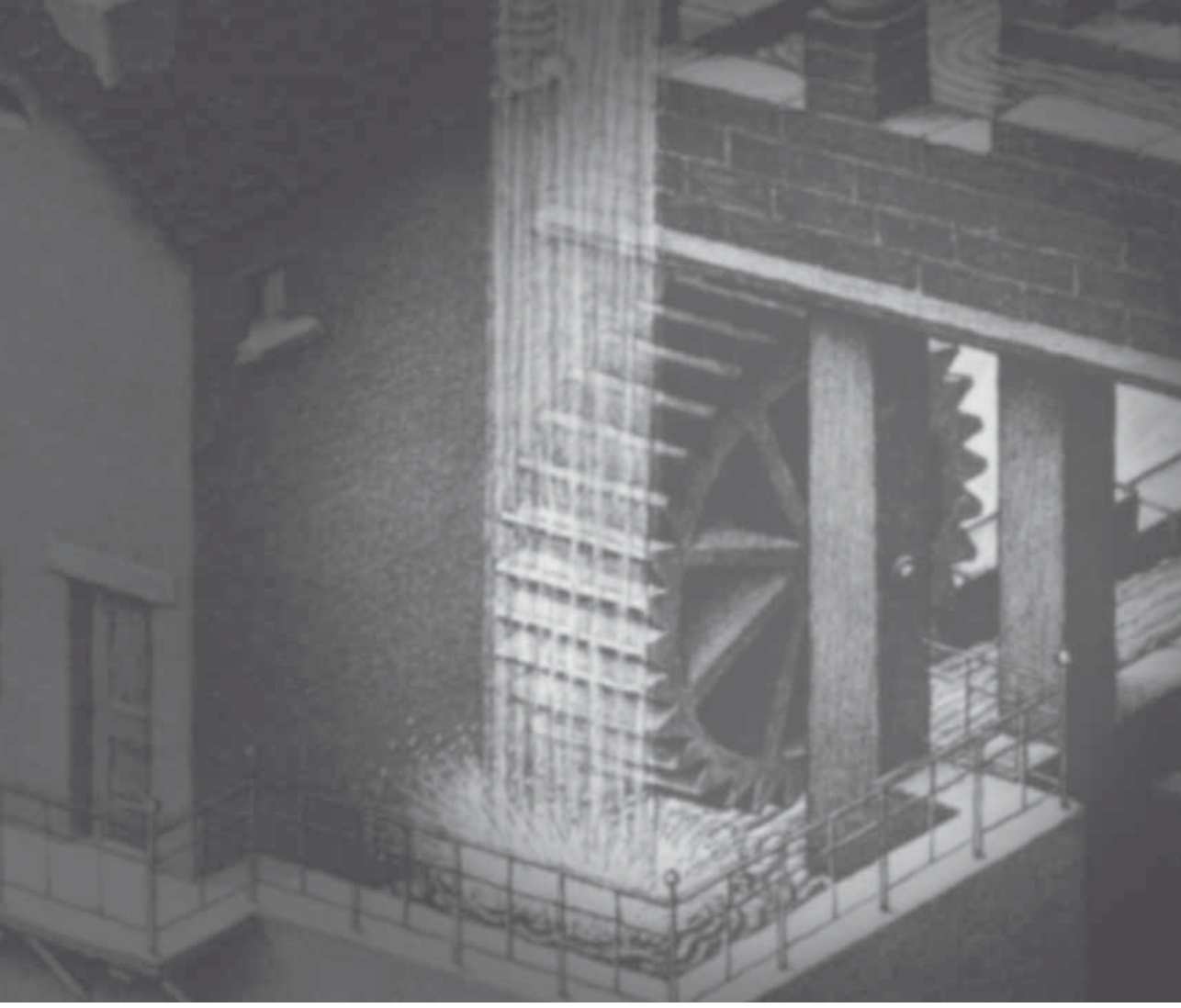
The frequency of FGFR3 mutations in the pTa and pT1mic tumours were respectively 78% and 63% (data not shown). We could speculate that pT1mic is similar to pTa. This could imply that distinction of pTa and pT1mic UC would not be of relevance. However the progression rate in 131 patients with pTa was substantially lower than in patients with pT1mic.

If confirmed in a larger series of pT1 UC, preferably in a prospective study, the sub-staging of papillary pT1 UC in pT1mic and pT1ext could be performed routinely. It provides the urologist additional information to identify patients with more aggressive tumours, to optimize therapy and adjust intensive surveillance.

References

1. Epstein JI, Amin MB, Reuter VR, et al: The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder. Bladder Consensus Conference Committee. Am J Surg Pathol 22:1435, 1998
2. Bryan RT, Wallace DM: 'Superficial' bladder cancer - time to uncouple pT1 tumours from pTa tumours. BJU Int 90:846, 2002
3. Kern WH: The grade and pathologic stage of bladder cancer. Cancer 53:1185, 1984
4. Amin MB, Gomez JA, Young RH: Urothelial transitional cell carcinoma with endophytic growth patterns: a discussion of patterns of invasion and problems associated with assessment of invasion in 18 cases. Am J Surg Pathol 21:1057, 1997
5. Parmar MK, Freedman LS, Hargreave TB, et al: Prognostic factors for recurrence and followup policies in the treatment of superficial bladder cancer: report from the British Medical Research Council Subgroup on Superficial Bladder Cancer (Urological Cancer Working Party). J Urol 142:284, 1989
6. Bakkar AA, Wallerand H, Radvanyi F, et al: FGFR3 and TP53 gene mutations define two distinct pathways in urothelial cell carcinoma of the bladder. Cancer Res 63:8108, 2003
7. Amling CL, Thrasher JB, Frazier HA, et al: Radical cystectomy for stages Ta, Tis and T1 transitional cell carcinoma of the bladder. J Urol 151:31, 1994
8. Zhang GK, Uke ET, Sharer WC, et al: Reassessment of conservative management for stage T1N0M0 transitional cell carcinoma of the bladder. J Urol 155:1907, 1996
9. Holmang S, Andius P, Hedelin H, et al: Stage progression in Ta papillary urothelial tumors: relationship to grade, immunohistochemical expression of tumor markers, mitotic frequency and DNA ploidy. J Urol 165:1124, 2001
10. van Rhijn BW, Vis AN, van der Kwast Th, et al: Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. J Clin Oncol 21:1912, 2003
11. Masters JR, Vani UD, Grigor KM, et al: Can p53 staining be used to identify patients with aggressive superficial bladder cancer? J Pathol 200:74, 2003
12. Lamina propria microinvasion of bladder tumors, incidence on stage allocation (pTa vs pT1): recommended approach. Pathologists of the French Association of Urology Cancer Committee. World J Urol 11:161, 1993
13. Younes M, Sussman J, True LD: The usefulness of the level of the muscularis mucosae in the staging of invasive transitional cell carcinoma of the urinary bladder. Cancer 66:543, 1990
14. Bol MG, Baak JP, Buhr-Wildhagen S, et al: Reproducibility and prognostic variability of grade and lamina propria invasion in stages Ta, T1 urothelial carcinoma of the bladder. J Urol 169:1291, 2003
15. van Rhijn BW, van der Kwast Th, Vis AN, et al: FGFR3 and P53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. Cancer Res 64:1911, 2004
16. Samaratunga H, Makarov DV, Epstein JI: Comparison of WHO/ISUP and WHO classification of noninvasive papillary urothelial neoplasms for risk of progression. Urology 60:315, 2002
17. Vis AN, Noordzij MA, Fitoz K, et al: Prognostic value of cell cycle proteins p27(kip1) and MIB-1, and the cell adhesion protein CD44s in surgically treated patients with prostate cancer. J Urol 164:2156, 2000
18. Billerey C, Chopin D, Aubriot-Lorton MH, et al: Frequent FGFR3 mutations in papillary non-invasive bladder (pTa) tumors. Am J Pathol 158:1955, 2001
19. Cappellen D, De Oliveira C, Ricol D, et al: Frequent activating mutations of FGFR3 in human bladder and cervix carcinomas. Nat Genet 23:18, 1999
20. van Rhijn BW, Montironi R, Zwarthoff EC, et al: Frequent FGFR3 mutations in urothelial papilloma. J Pathol 198:245, 2002
21. McKenney JK, Gomez JA, Desai S, et al: Morphologic expressions of urothelial carcinoma in situ: a detailed evaluation of its histologic patterns with emphasis on carcinoma in situ with microinvasion. Am J Surg Pathol 25:356, 2001
22. Zincke H, Utz DC, Farrow GM: Review of Mayo Clinic experience with carcinoma in situ. Urology 26:39, 1985

23. Holmang S, Hedelin H, Anderstrom C, et al: The importance of the depth of invasion in stage T1 bladder carcinoma: a prospective cohort study. J Urol 157:800, 1997
24. Cheng L, Weaver AL, Neumann RM, et al: Substaging of T1 bladder carcinoma based on the depth of invasion as measured by micrometer: A new proposal. Cancer 86:1035, 1999
25. Platz CE, Cohen MB, Jones MP, et al: Is microstaging of early invasive cancer of the urinary bladder possible or useful? Mod Pathol 9:1035, 1996



PART III

SURVEILLANCE IN CLINICAL PRACTICE

CHAPTER 6

Microsatellite Analysis for surveillance of bladder cancer

CHAPTER 7

Cystoscopy revisited

CHAPTER 6

Microsatellite Analysis of voided urine samples for surveillance of low-grade non-muscle invasive urothelial carcinoma: feasibility and clinical utility in a prospective multicenter study (CEFUB)

Madelon N.M. van der Aa
Ellen C. Zwarthoff
Ewout W. Steyerberg
Merel W. Boogaard
Yvette Nijsen
Kirstin A van der Keur
Antonius J. A. van Exsel
Wim J. Kirkels
Chris Bangma
Theo H. van der Kwast

Abstract

Microsatellite analysis (MA) on voided urine samples has been promoted as an alternative for cystoscopy surveillance (UCS) of patients with low-grade non-muscle invasive papillary urothelial carcinoma (UC). To assess the feasibility and clinical utility of microsatellite analysis (MA) on voided urine samples in a routine setting to detect or predict bladder cancer recurrences. We evaluated 228 patients monitored by MA on voided urine samples and synchronous cystoscopic examination, who participated in a longitudinal prospective study in 10 hospitals. Follow-up started after diagnosis of a primary or recurrent pTa, pT1, grade 1 or 2 papillary UC. Clinico-pathological parameters and FGFR3 mutation status of the inclusion tumour were determined. MA outcome was analyzed in 1012 urine samples during a mean follow-up of 41 months. Poor DNA quality prevented MA in 19% (197/1012) of the samples, leaving 815 visits for a cross-sectional analysis of sensitivity and specificity. We determined the predictive value (PPV) in a longitudinal analysis for 458 series with persistent MA results. Factors influencing diagnostic quality of MA were investigated. Kaplan Meier analysis was performed to relate MA results to recurrence. Cross-sectional sensitivity and specificity of MA for detection of a recurrence was 58% (49/84) and 73% (531/731), respectively. One pT1 grade 3 UC was missed. In a longitudinal analysis, the 2-year risk to develop a recurrence reached 83% if MA outcome was persistently positive and 22% when persistently negative. PPV of MA was higher with wild type FGFR3 gene status and smoking habits. All 4 upper urinary tract tumours detected were preceded by a positive MA test. Consecutive positive MA results are a strong predictor for future recurrences, but sensitivity needs to be improved, e.g. by patient selection and testing of additional genetic markers in urine samples.

Introduction

Bladder cancer is the fifth most common malignancy in Europe and accounts for 5% of all diagnosed cancers[1]. In 80% of the patients the bladder tumour will primarily appear as a papillary non-muscle invasive (stage pTa and pT1) urothelial carcinoma (NMI UC). Up to 70% of these tumours will recur and 15% will progress in stage or grade[2]. Therefore patients require extensive surveillance, currently based on cystoscopy and cytology. Although cystoscopy is a reliable method, it is an invasive and costly procedure[3]. Several studies reported on the use of microsatellite analysis (MA) for detection of cells with loss of heterozygosity (LOH) in voided urine samples as a tool for detection of recurrent UC in the urinary bladder and in the upper urinary tract[4-7]. For a new method to compete with cystoscopy it needs to be feasible in routine clinical practice: highly reproducible and accurate at acceptable costs. Moreover, sensitivity needs to be high, since 89% of the patients would prefer flexible cystoscopy if the sensitivity of a urinary test were lower than 90%[8]. One of the most promising prognosticators of NMI UC is the mutation status of the fibroblast growth factor receptor 3 (FGFR3)[9-12]. This mutation is associated with genetically stable bladder cancer and an indicator for favourable prognosis[13]. We therefore studied the impact of the FGFR3 gene mutation status on the performance of MA. In our Cost-effectiveness of Follow-up of Urinary Bladder Cancer (CEFUB) trial we aimed to assess the feasibility and clinical utility of MA on voided urine samples in a routine clinical practice in a prospective multicenter study on a large patient group with low-grade NMI UC. We used cross-sectional analysis to assess the diagnostic value of a MA result at a single follow-up visit. Furthermore we performed a longitudinal analysis to reflect the positive predictive value (PPV) of a positive MA result preceding a cystoscopically visible tumour.

Materials and Methods

Study design

A total of 448 patients with NMI UC (i.e. TNM 1997 stage pTa, pT1, and WHO 1973 grade 1 or 2) were recruited by 10 Dutch hospitals for participation in the randomized CEFUB trial after signing an informed consent form (registration: ClinicalTrials.gov NCT00126958). They were randomized after resection of NMI UC for a follow-up either by 3-monthly cystoscopy alone (control arm) or by cystoscopy at 3 months, 12 months and 24 months and 3-monthly MA on voided urine samples (test arm). Inclusion of patients in the CEFUB-trial was from June 2002 until June 2006 and their follow-up by MA was ended in November 2006. During the first year of the study MA was also performed in the control arm, but the results were not communicated to the urologist. For the test arm, the protocol recommended imaging of the upper urinary tract if positive MA outcome was incongruent with cystoscopy during

two follow-up visits. Patients with a history of carcinoma in situ or grade 3 UC were excluded from participation. The study presented in this publication concerns 228 participants (UCS arm n= 102, MA arm n= 126) of both trial arms whose cystoscopy and synchronous MA data were both available in 1012 follow-up visits. Follow-up ranged between 16 and 71 months (mean time of follow-up 41 months). The ethical committees of the participating institutions approved the study.

Sample Collection

At the outpatient clinics of the 10 participating hospitals 10 ml heparinized whole venous blood and urine samples (15-100ml) were collected and stored at +4°C until transportation at +4°C by courier service to the department of Pathology Erasmus MC, Rotterdam. DNA from the blood samples was isolated using the DNeasy Blood Kit (Qiagen GmbH, Hilden Germany). Histopathological reports along with the corresponding HE-slides and paraffin blocks were submitted to Erasmus MC for determination of the FGFR3 mutation status. A pathological review was not performed. Freshly voided urine was collected before cystoscopy. Time between collection of urine and arrival at the molecular laboratory at the Erasmus MC, Rotterdam varied between 1 and 240 hours (median 24 hours). If the concentration of leukocytes in the urine samples was > 20 leukocytes per microscopic field (magnification x 400) the patient was asked to collect a urine sample again. Urine samples were washed twice with phosphate-buffered saline (PBS). The final pellet was resuspended in 1.0 mL PBS and stored at -20 °Celsius until DNA isolation. The QIAamp Viral RNA kit (Qiagen GmbH, Hilden, Germany) was used, according to the manufacturers protocol, for isolation of DNA. Isolation finally provided an 80-µl sample of DNA product. Clinical data of follow-up results of cystoscopy, additional diagnostics and (intra-vesical) therapy of these patients were obtained from standardized case record forms.

Microsatellite Analysis

DNA from each urine sample was amplified by PCR using primers for 20 polymorphic microsatellite markers localized on 10 chromosomes[4, 6, 7]. PCR amplification was carried out in PCR buffer (Promega, Madison, WI) and FAM fluorescent-labelled primers (Applied Biosystems, Foster City, CA; range of primer concentration 0.2-1.75 pM). Amplified fragments were analysed by using the ABI 3100 Genetic Analyser. LOH was defined: the ratio of allelic loss as quantified by calculation of the blood/urine-ratio (i.e. peak height in blood (allele 1/ allele 2): peak height in urine (allele 1/ allele2). LOH was established when 0.7 > blood/urine ratio > 1.42. All LOHs were confirmed by a second independent polymerase chain reaction (PCR). LOH of ≥ 1 marker is sufficient for diagnosis of LOH. The peak height ratio of blood-determined alleles was the average of the ratios of 4 independent PCRs separate for each

marker. A marker was excluded for a patient when its standard deviation was ≥ 15%. We applied a lower limit for peak heights of 700 to exclude possible preferential amplification of one of the two alleles due to insufficient input DNA. All reactions with off-scale signals were diluted and rerun. A result was only taken into account when at least 10 MA markers in a sample were informative. The laboratory analyses were performed without knowledge of clinical or histological status.

Molecular analysis for *FGFR3* mutation

Tissue samples used for *FGFR3* mutation analysis contained a minimum of 70% tumour cells, as assessed by histological examination. DNA from tumour and control DNA from venous blood samples of all patients was extracted using the DNeasy Tissue Kit (Qiagen GmbH, Hilden Germany). Exons 7, 10 and 15, encompassing the 11 activating *FGFR3 gene* mutations most commonly involved with UC were amplified by PCR. A detailed description of *FGFR3* mutation analysis was given previously[14]. The laboratory analyses were performed without knowledge of clinical or histopathological status.

Statistical Analysis

All data was compiled into a Microsoft Access database. Descriptive and statistical analyses were performed using SPSS 11.5 software (SPSS Inc., Chicago, Ill). Recurrence was defined by histological diagnosis of UC. Sensitivity and specificity were determined by cross-sectional analysis for every follow-up visit with synchronous cystoscopy and MA results. PPV was determined by longitudinal analysis of one or more subsequent follow-up visits (MA series) with either positive MA outcomes (persistent positive MA) or negative MA outcomes (persistent negative MA). A series of MA was thus defined as one or more consecutive positive or negative MA outcomes ending at the time of 1) a recurrence, including UUT, 2) a change in MA outcome from positive to negative or from negative to positive or 3) at end of further follow-up. In case of a NMI UC recurrence or a change of MA outcome, a new series would start for this patient. Recurrences in longitudinal series were analysed by the Kaplan-Meier method and compared using the log-rank test. $P < 0.05$ was considered statistically significant.

Results

Patients, tumours and voided urine samples

Table 6.1 shows the clinical features of the 228 patients, as well as the histopathological and molecular tumours characteristics. Patients from both trial arms were evaluated (test arm, n=126; control arm, n=102), if matched MA and cystoscopy results were available. The 3-monthly surveillance provided 1012 urine samples. In 197/1012 (19%) urine samples the MA test failed, in 11 samples due to technical failure and in 186 samples no detectable signal

Table 6.1

Clinical features in 228 patients							
Disease status at start of study		Primary n=118	1st recurrence n=45	2nd recurrence n=30	3rd recurrence n=16	≥4th recurrence n=19	Total n= 228
Patient characteristics							
Age, mean ± sd	63y ± 12						
Gender	Male	91 (77%)	34 (77%)	22 (71%)	13 (81%)	14 (74%)	174 (76%)
Smoking behaviour	Smoker	77 (76%)	28 (78%)	19 (73%)	6 (50%)	13 (81%)	143 (75%)
Tumor characteristics at start of study of 228 patients							
Stage	pTa	100 (85%)	38 (86%)	26 (84%)	15 (94%)	16 (84%)	195 (86%)
	pT1	18 (15%)	6 (14%)	5 (16%)	1 (6%)	3 (16%)	33 (14%)
Grade	G1	51 (43%)	23 (52%)	16 (52%)	7 (44%)	12 (63%)	109 (48%)
	G2	67 (57%)	21 (48%)	15 (48%)	9 (56%)	7 (37%)	119 (52%)
FGFR3	Mutant type	60 (61%)	26 (67%)	20 (74%)	12 (80%)	11 (61%)	129 (65%)
	Wild type	38 (39%)	13 (33%)	7 (26%)	3 (20%)	7 (39%)	68 (35%)
Focality	Solitary	77 (73%)	24 (60%)	12 (40%)	10 (67%)	9 (64%)	132 (64%)
	Multifocality	29 (27%)	16 (40%)	18 (60%)	5 (33%)	5 (36%)	73 (36%)

Characteristics of 228 Patients and their Tumors at Start of Study Surveillance

was observed. This was attributable to prolonged periods of storage between moment of sampling and processing (Figure 6.1a). Figure 6.1b demonstrates that up to about 6 months after intravesical chemo/immunotherapy the MA test failed slightly more often in treated versus untreated patients during the same time period. Mean time of reporting of MA to the urologist after requisition of the sample was 10 working days (range 4-33 days).

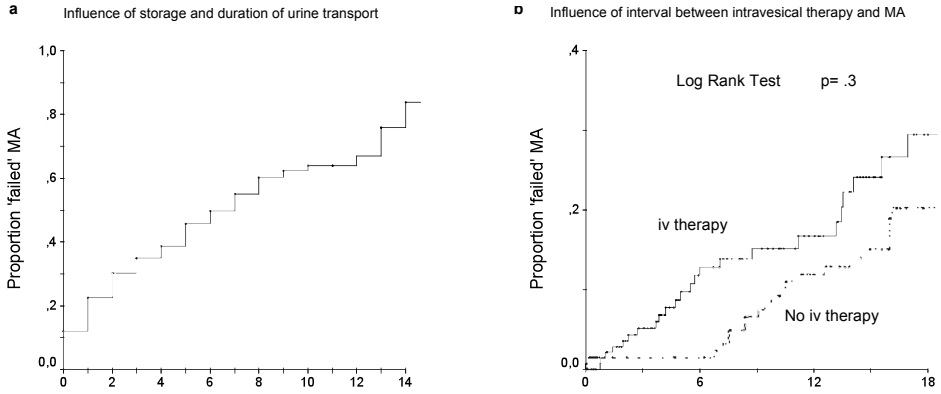
Quality of microsatellite markers

All microsatellite markers were performing well. Few LOHs were found with D4S242, FGA, D11S488, D17S960, D18S51 and D20S454. Most LOHs were found with the markers from chromosome 9. D9S171 revealed fewer LOHs than other 9p markers, probably because its location is in the vicinity of the p16 gene in which homozygous deletions often occur in bladder cancer. We further noticed that tetranucleotide repeat markers were easier to work with than CA-repeat-containing markers due to fewer stutter peaks.

Sensitivity and specificity of MA for detection of recurrences: cross-sectional analysis

A total of 815 follow up visits with both MA data and synchronous cystoscopy findings could be analysed for 84 detected histologically proven recurrences (Table 6.2). The sensitivity, specificity of the MA test was 58% (49/84), 73% (531/731) respectively. If our analysis was confined to the patients of the MA arm, a sensitivity of 70% (42/60) of the MA test was found. Table 6.3 describes the characteristics of the patients and tumours at inclusion that developed a recurrence during follow-up of the trial and compares the clinico-pathological and

Figure 6.1



Prolonged duration between urine collection and MA and intravesical therapy negatively affect quality of MA in 19% (197/1012) of the samples.
a. Effect of time till analysis of urine sample. After 2 weeks of delay MA failed in 80%.
b. Effect of intra-vesical immune/chemotherapy (iv therapy). In the first 6 months after intra-vesical therapy MA of 15% of urine samples failed, compared to 8% in samples from patients without iv therapy. A total of 88 patients was treated with intravesical therapy after TUR.

Table 6.2

MA outcome	UC recurrence	No UC recurrence	Total
MA +	49	200	249
MA -	35	531	566
Total	84	731	815

Sensitivity	58% (49/84)	95% CI [0.47-0.69]
Specificity	73% (531/731)	95% CI [0.69-0.76]
Positive Predictive Value	20% (49/200)	95% CI [0.15-0.25]
Negative Predictive Value	94% (531/566)	95% CI [0.92-0.96]

MA outcome and histologically proven recurrences detected at synchronous cystoscopy findings during 815 follow-up visits.

molecular characteristics of the recurrences detected and missed by MA. The recurrences missed by MA are predominantly pTa Grade 1 and 2 UC, but they include also one pT1 Grade 3 UC. On the other hand, three cases of CIS, all four ≥ pT2 UC recurrences and 7 of 8 recurrent Grade 3 cancers were detected in the MA positive group.

MA as a predictor for future bladder cancer recurrences; a longitudinal analysis

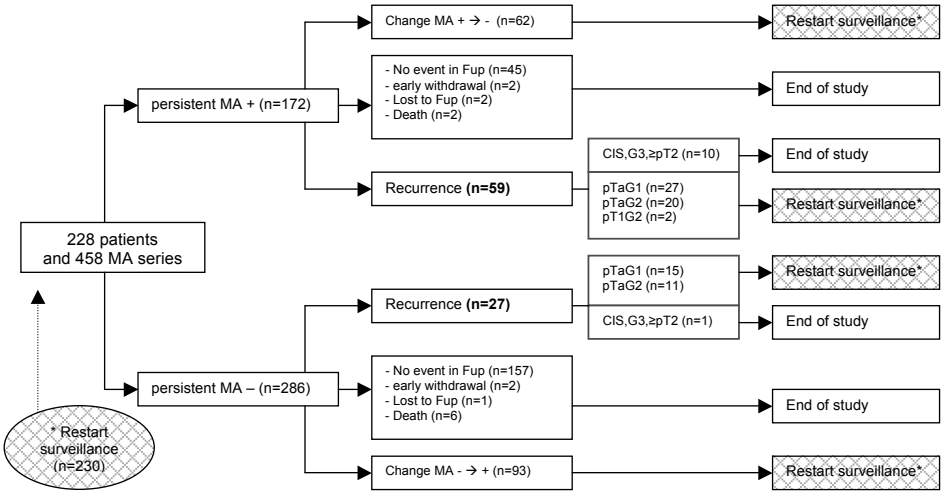
To appreciate the predictive value of MA for the detection of future recurrences we performed a longitudinal analysis on a total of 458 series of MA outcomes from 228 patients with a median follow-up of 34 months. The flowchart (Figure 6.2) shows the possible endpoints and the accompanying numbers of events during follow-up. In this longitudinal analysis 86 recurrences were detected including 4 upper urinary tract tumours, identified after a positive MA test by radio-diagnostic imaging (X-ray, CT scan or intravenous urogram). Kaplan Meier analyses (Figure 6.3) showed that patients with consecutive series of persistently MA positive

Table 6.3

Characteristics of 57 patients and tumours at inclusion developing recurrences			
Gender	Male	42 (74%)	
Randomised Groups	Control Arm (UCS)	18 (32%)	
Smoking	Yes	35 (70%)	
Stage	pTa	47 (82%)	
Grade	G1	28 (49%)	
Multifocality	Multifocal	21 (44%)	
FGFR3 at inclusion	Mutant	31 (60%)	
Characteristics of 84 recurrences		Detection of recurrences by MA	
		Yes (%)	No (%)	Univariate p value
Stage	pTa	38 (53)	34 (47)	
	pT1	7 (88)	1 (12)	
	≥pT2	4 (100)	0 (0)	
Concomitant CIS	CIS	3 (100)	0 (0)	0.03
Grade	G1	24 (57)	18 (43)	
	G2	16 (50)	16 (50)	
	G3	9 (90)	1 (10)	0.4
Multifocality	Multifocal	20 (54)	17 (46)	
	Solitary	24 (62)	15 (38)	0.2

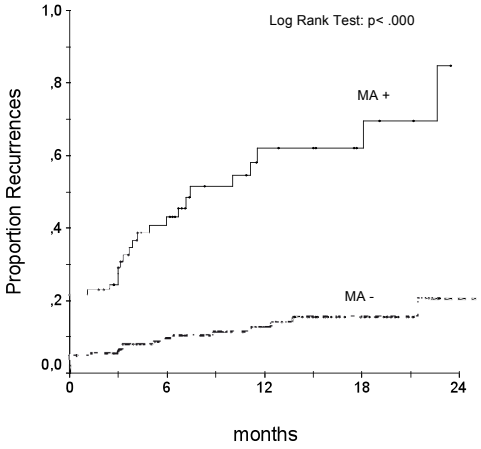
Clinicopathological and molecular features of 84 recurrences in 57 patients related to synchronous MA findings.

Figure 6.2



Flowchart showing the possible endpoints after completion of a MA series and the accompanying numbers of events. Endpoints were final (end of study or progressive recurrence) or temporary (change of MA result or NMI-UC). Temporary endpoints and series ending with a histologically proven non-muscle invasive recurrence re-started a new MA series (*).

Fgure 6.3



Kaplan Meier Curve shows the proportion of recurrences in 458 MA events detected by consecutive continuous positive or negative results of MA. Time (in months) reflects the length of time of continuous MA results without visible suspicious lesion in the bladder. After 24 months, 83% of recurrences is detected, 22% is missed.

follow-up visits had an 83% risk of recurrence after 24 months (59 recurrences predicted). In contrast, after a series of one or more persistently negative MA tests the probability to miss a developing recurrence in 24 months was 22% (27 recurrences missed, in 21 patients). Series of positive MA tests preceded recurrences by 1 to 24 months (Figure 6.4).

Figure 6.4

Time (months)	D9S242	D9S252	D16S476	D17S786	D17S960	D18S51	D20S454	MA	Cysto scopy	Policy	Diagnosis
3	0.880	1.064	1.060	NP	NP	0.981	1.630	LOH +	Neg	X-IVP	No abnormality
6	0.666	1.127	1.112	1.857	0.486	0.892	1.533	LOH +	Neg	C/3	-
9	0.675	1.233	0.836	2.661	0.429	1.025	1.701	LOH +	Pos	TUR	pT1G2
12	0.927	0.928	1.168	1.155	0.800	0.974	1.205	LOH -	Neg	C/3	-
15	0.916	1.024	0.998	1.202	0.783	1.003	1.225	LOH -	Neg	C/3	-
18	0.919	0.958	0.903	1.410	0.544	0.978	1.379	LOH +	Neg	C/3	-
21	0.851	1.077	0.954	4.514	0.104	1.006	1.763	LOH +	Neg	X-IVP	No abnormality
24	1.033	NP	0.807	12.135	0.040	1.074	2.021	LOH +	Pos	TUR	pTaG2

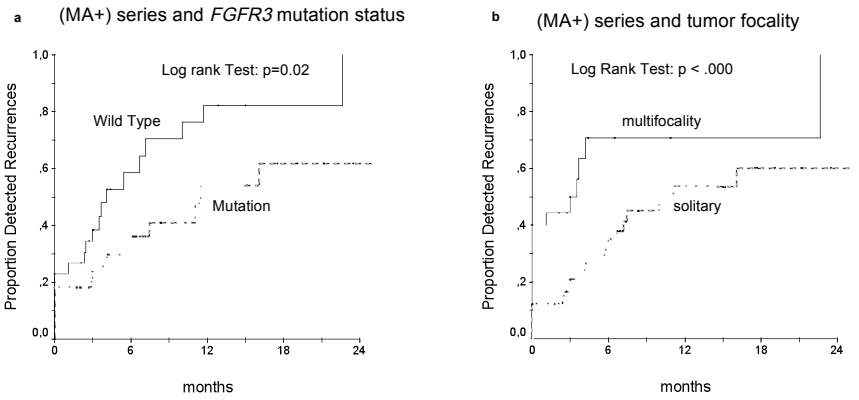
Example of follow-up by cystoscopy and MA:

Female, 62 years at time of primary tumor, non smoker. History: June 2000 Nephrectomy, pTaG2; Nov 2000 primary UC Bladder, pT1G2; April 2002 3 recurrences in bladder, pTaG2, wt (*FGFR3 gene* mutation status). Time is in months after inclusion. D9S242, D9S252, D16S476, D17S786, D17S960, D18S51 and D20S454 are MA markers quantified by NP (No DNA product) or by the ratio of allelic loss as quantified by calculation of the blood/urine-ratio (LOH was established when 0.7 > blood/urine ratio > 1.42); MA results are (LOH+) suspect for UC or (LOH-) not suspect; cystoscopy shows lesion suspect for tumor (Pos) or no visual lesions (Neg); clinical policy could be Trans Urethral Resection (TUR), outpatient clinic visit in 3 months (C/3) or adjuvant imaging (X/IVP or CTscan).

Parameters influencing positive predictive value of MA

The predictive value of MA was considerably higher in patients included with a FGFR3 wild type tumour (24/31, 77%) when compared to patients with a mutant tumour (28/46, 61%). FGFR3 mutation status of the initial tumour, tumour focality, (Figure 6.5) and gender and smoking habits (Figure 6.6 and 6.7) all revealed significant differences with regard to detection of recurrences by MA.

Figure 6.5

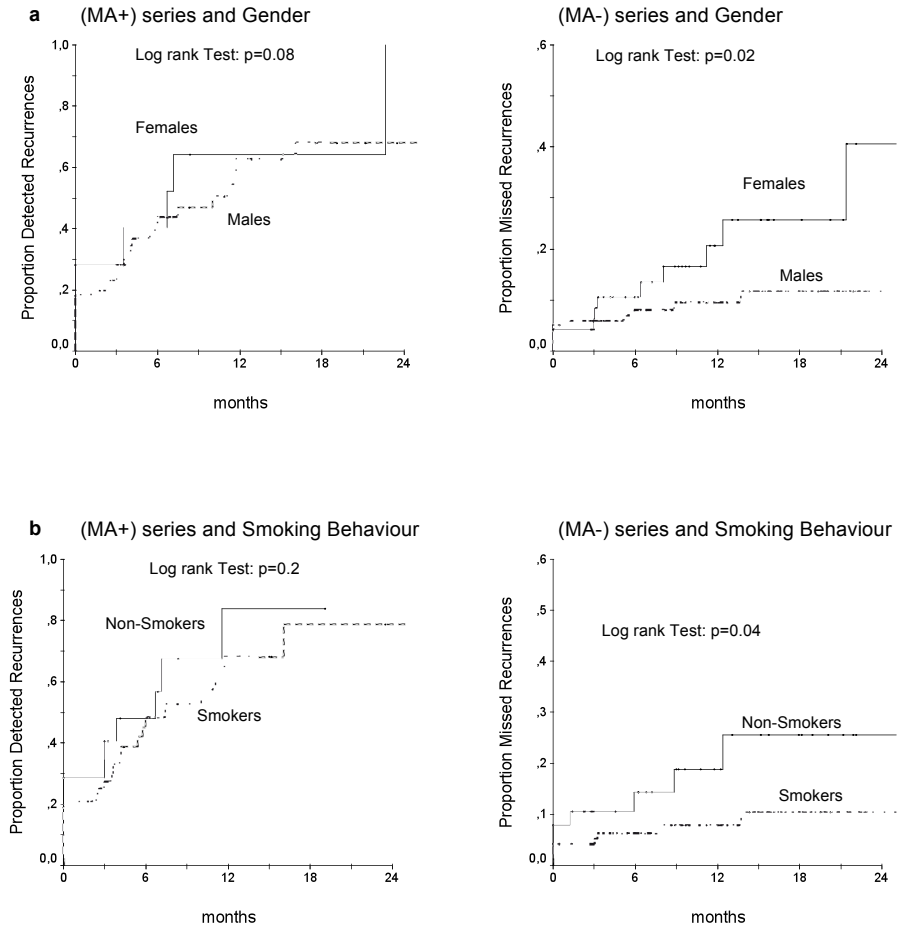


Kaplan-Meier curves relating FGFR3 mutation status (a) and tumour multifocality (b) at entry to MA detected recurrences.

Discussion

This prospective study was designed to evaluate the feasibility and clinical utility to perform MA on voided urine samples derived from different hospitals in a central molecular-genetic laboratory. Although the vast majority of urine samples (81%) were of sufficient quality to allow MA, transportation times longer than 48 hours to the central laboratory adversely affected the proportion of samples giving satisfactorily results. Thus, adequate logistics are required prior to implementation of MA testing in a routine setting. The main finding of our study is the relatively low cross-sectional sensitivity 58% (49/84) and specificity 73% (531/731) of MA on voided urine samples to detect recurrences. A potential limitation of our study is that our main analysis is based on the combined data from two trial arms, one of which comprised the unblinding of the urologist for the MA outcome. It is conceivable that awareness of a positive MA outcome may have prompted a higher detection rate of UC recurrences by cystoscopy. Indeed, separate analysis of the patients of the MA arm showed an improved cross-sectional sensitivity (70%) of the MA test. From the perspective of cost-effectiveness it must be noted that our semi-automated high-throughput MA is a labour-intensive technique and its routine application can be improved by a better selection of markers and a further automatization

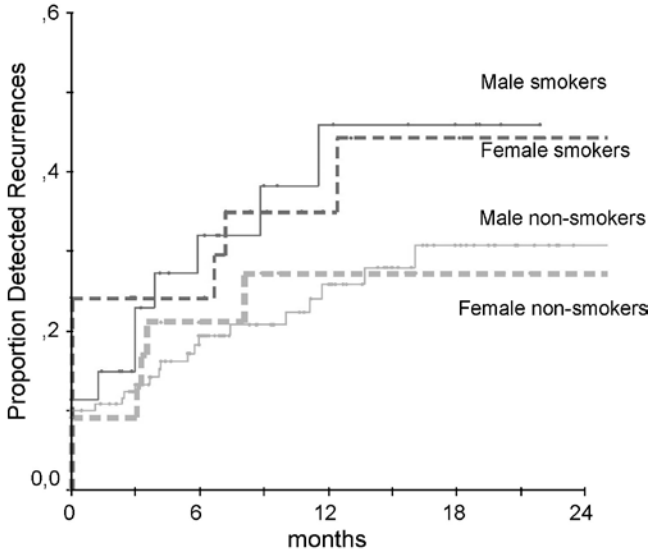
Figure 6.6



Kaplan Meier curves relating gender (a), smoking habits (b), FGFR3 mutation status of the tumour (c) and tumour focality at entry (d) to MA detected recurrences.

of the procedure[15, 16]. In a meta-analysis on urine markers it was reported that the sensitivity and specificity of MA during follow-up of patients with UC varies between 75-92% and 79-100% respectively[17]. Contrary to these latter studies, our series was entirely composed of grade 1 and 2 NMI UC. These patients may especially benefit from surveillance tests other than cystoscopy. MA is more sensitive for detection of higher stage and grade recurrences, although one T1G3 tumour was missed. Since the WHO 1973 grade 2 UC comprise a considerable proportion of High-grade UC according to the more recent WHO/ISUP 2004 grading system, it follows that MA misses a larger number of the latter category of UC. Longitudinal analysis revealed that the relatively low specificity of 73% was mainly due to positive MA outcomes preceding a cystoscopically visible recurrence by many months. We showed in an earlier study that MA can anticipate the occurrence of recurrences[7]. Other urine tests also

Figure 6.7



Kaplan Meier curves relating gender and smoking habits demonstrate that smoking habits determine the proportion of MA detectable recurrences.

demonstrated a better prediction of UC recurrence during longitudinal follow-up (18). Amira et al. were the first who analysed subsequent MA results in a systematic study design[19]. They found that a positive MA test preceded a visible recurrence between 1 month and 9 months in 75% of patients. Similarly, our study of NMI UC demonstrates that the PPV was 83% for 24 months of follow-up with persistent positive MA results. Our findings also confirmed previous studies that MA is able to detect upper urinary tract UC[4, 7, 20]. Multiple subsequent positive or negative MA tests are important for the likelihood of detecting a recurrence. We hypothesize that the missed recurrences were mainly genetically stable UC (pTa, low-grade).

The positive predictive value of MA is much higher in patients with an FGFR3 wild type tumour reaching 100% in 24 months (Fig. 6.5a). This is in line with the finding that FGFR3 gene mutations are associated with genetically more stable UC[21]. We also noted that MA misses more recurrences in non-smoking patients and surveillance by MA could be especially valuable for smoking patients with an FGFR3 wild type tumour. The somewhat enhanced sensitivity of MA to detect UC recurrences in patients with smoking habits may be explained by their propensity to develop genetically more unstable bladder cancers[22]. For patients with an FGFR3 mutant tumour, surveillance by FGFR3 mutation analysis could be envisaged[14, 23] .

The FDA approved UroVysion FISH test is a methodologically different test, but is also based on the detection of genetic abnormalities in cells shed in urine. In a recent paper on 250 patients, including patients with a high-grade UC using UroVysion on cytological specimens of voided urine, the predictive rather than diagnostic capacity of this test (sensitivity 60%) was emphasized[24]. In this study three high-grade, high stage cancers were missed. In 35/56

(63%) patients with a positive UroVysion test, the cystoscopy became positive at a later time during a total follow-up period of 29 months.

Our findings in NMI UC are thus very much in line with 1) the somewhat lower than expected cross-sectional sensitivity of these genetic tests (MA and UroVysion-FISH)[25] and 2) the evolving concept that genetic changes detected in urine samples often precede the cystoscopically detectable recurrence by several months[26].

Conclusion

A positive MA test is a strong predictor for future recurrences. Testing of additional genetic markers in urine samples and stratification of NMI UC and patients based on genetic instability may improve accuracy of MA. However, MA on voided urine samples is currently not sufficiently sensitive to recommend implementation in routine clinical practice.

References

1. Sengupta N, Siddiqui E, Mumtaz FH. Cancers of the bladder. J R Soc Health 2004;124:228-9.
2. Raghavan D, Shipley WU, Garnick MB, Russell PJ, Richie JP. Biology and management of bladder cancer. N Engl J Med 1990;322:1129-38.
3. Mariappan P, Smith G. A surveillance schedule for G1Ta bladder cancer allowing efficient use of check cystoscopy and safe discharge at 5 years based on a 25-year prospective database. J Urol 2005;173:1108-11.
4. Mao L, Schoenberg MP, Scicchitano M, Erozan YS, Merlo A, Schwab D, et al. Molecular detection of primary bladder cancer by microsatellite analysis. Science 1996;271:659-62.
5. Seripa D, Parrella P, Gallucci M, Gravina C, Papa S, Fortunato P, et al. Sensitive detection of transitional cell carcinoma of the bladder by microsatellite analysis of cells exfoliated in urine. Int J Cancer 2001;95:364-9.
6. Steiner G, Schoenberg MP, Linn JF, Mao L, Sidransky D. Detection of bladder cancer recurrence by microsatellite analysis of urine. Nat Med 1997;3:621-4.
7. Van Rhijn BW, Lurkin I, Kirkels WJ, Van Der Kwast Th, Zwarthoff EC. Microsatellite analysis--DNA test in urine competes with cystoscopy in follow-up of superficial bladder carcinoma: a phase II trial. Cancer 2001;92:768-75.
8. Vriesema JL, Poucki MH, Kiemeney LA, Witjes JA. Patient opinion of urinary tests versus flexible urethrocystoscopy in follow-up examination for superficial bladder cancer: a utility analysis. Urology 2000;56:793-7.
9. Van Rhijn BW, Vis AN, Van Der Kwast Th, Kirkels WJ, Radvanyi F, Ooms EC, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. J Clin Oncol 2003;21:1912-21.
10. Hernandez S, Lopez-Knowles E, Lloreta J, Kogevinas M, Amoros A, Tardon A, et al. Prospective study of FGFR3 mutations as a prognostic factor in nonmuscle invasive urothelial bladder carcinomas. J Clin Oncol 2006;24:3664-71.
11. Van Oers JM, Wild PJ, Burger M, Denzinger S, Stoehr R, Rosskopf E, et al. FGFR3 mutations and a normal CK20 staining pattern define low-grade noninvasive urothelial bladder tumours. Eur Urol 2007;52:760-8.
12. Burger M, Van Der Aa MN, Van Oers JM, Brinkmann A, Van Der Kwast Th, Steyerberg EC, et al. Prediction of Progression of Non-Muscle-Invasive Bladder Cancer by WHO 1973 and 2004 Grading and by FGFR3 Mutation Status: A Prospective Study. Eur Urol 2007;
13. Knowles MA. Role of FGFR3 in urothelial cell carcinoma: biomarker and potential therapeutic target. World J Urol 2007;25:581-93.
14. Van Oers JM, Lurkin I, Van Exsel AJ, Nijsen Y, Van Rhijn BW, Van Der Aa MN, et al. A simple and fast method for the simultaneous detection of nine fibroblast growth factor receptor 3 mutations in bladder cancer and voided urine. Clin Cancer Res 2005;11:7743-8.
15. Frigerio S, Padberg BC, Strebel RT, Lenggenhager DM, Messthaler A, Abdou MT, et al. Improved detection of bladder carcinoma cells in voided urine by standardized microsatellite analysis. Int J Cancer 2007;121:329-38.
16. Koed K, Wiuf C, Christensen LL, Wikman FP, Zieger K, Moller K, et al. High-density single nucleotide polymorphism array defines novel stage and location-dependent allelic imbalances in human bladder tumors. Cancer Res 2005;65:34-45.
17. Van Rhijn BW, Van Der Poel HG, Van Der Kwast Th. Urine markers for bladder cancer surveillance: a systematic review. Eur Urol 2005;47:736-48.
18. Van der Poel HG, van Rhijn BW, Peelen P, Debruyne FM, Boon ME, Schalken JA. Consecutive quantitative cytology in bladder cancer. Urology. 2000 ;56: 584-8.
19. Amira N, Mourah S, Rozet F, Teillac P, Fiet J, Aubin P, et al. Non-invasive molecular detection of bladder cancer recurrence. Int J Cancer 2002;101:293-7.
20. Eisenberger CF, Schoenberg M, Enger C, Hortopan S, Shah S, Chow NH, et al. Diagnosis of renal cancer by molecular urinalysis. J Natl Cancer Inst 1999;91:2028-32.
21. Van Rhijn BW, Lurkin I, Chopin DK, Kirkels WJ, Thiery JP, Van Der Kwast Th, et al. Combined microsatellite and FGFR3 mutation analysis enables a highly sensitive detection of urothelial cell carcinoma in voided urine. Clin Cancer Res 2003;9:257-63.

22. Wallerand H, Bakkar AA, De Medina SG, Pairon JC, Yang YC, Vordos D, et al. Mutations in TP53, but not FGFR3, in urothelial cell carcinoma of the bladder are influenced by smoking: contribution of exogenous versus endogenous carcinogens. Carcinogenesis 2005;26:177-84.
23. Rieger-Christ KM, Mourtzinos A, Lee PJ, Zagha RM, Cain J, Silverman M, et al. Identification of fibroblast growth factor receptor 3 mutations in urine sediment DNA samples complements cytology in bladder tumor detection. Cancer 2003;98:737-44.
24. Yoder BJ, Skacel M, Hedgepeth R, Babineau D, Ulchaker JC, Liou LS, et al. Reflex UroVysion testing of bladder cancer surveillance patients with equivocal or negative urine cytology: a prospective study with focus on the natural history of anticipatory positive findings. Am J Clin Pathol 2007;127:295-301.
25. Moonen PM, Merkx GF, Peelen P, Karthaus HF, Smeets DF, Witjes JA. UroVysion compared with cytology and quantitative cytology in the surveillance of non-muscle-invasive bladder cancer. Eur Urol 2007;51:1275-80; discussion 80.
26. Sarosdy MF, Schellhammer P, Bokinsky G, Kahn P, Chao R, Yore L, et al. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. J Urol 2002;168:1950-4.

CHAPTER 7

Cystoscopy revisited as the gold standard for detection of bladder cancer recurrences: diagnostic review bias in a randomized prospective trial (CEFUB-trial)

Madelon N.M. van der Aa
Ewout W. Steyerberg
Chris Bangma
Bas W.G. van Rhijn
Ellen C. Zwarthoff
Theo H. van der Kwast

Abstract

The aim of this study was to evaluate the influence of knowledge of a urine-test outcome on the accuracy of cystoscopy (diagnostic review bias) during surveillance of patients with low-grade non-muscle invasive urothelial carcinoma (NMI UC). Prospective, single blinded, randomized multicenter clinical trial evaluating the cost-effectiveness of surveillance by a urine test (i.e. microsatellite analysis) of 448 patients presenting with primary or recurrent NMI UC (pTa, pT1, G1, G2). Urine test results (positive or negative) were only communicated to the urologist in the intervention arm (n=226), where patients had cystoscopy if the test was positive and at 3, 12, and 24 months. Results were not communicated in the control arm (n=222) with standard 3-monthly cystoscopy. Primary outcome measure was the number of histologically proven bladder cancer recurrences. : During a median follow-up of 34 months, 218 recurrences were detected in the intervention arm, compared to 163 in the control arm ($p<0.001$). In 131 cystoscopies performed with knowledge of a positive urine test, 42 recurrences were detected. Only 6 recurrences were found for the 120 cystoscopies without information on the positive test result (chi-square $p< 0.001$). There was no difference in detection of recurrences when urine test results were negative (intervention arm: 18/260 (7%), control arm 18/326 (6%), $p=0.45$).

Awareness of a positive urine test result significantly improves the UC detection rate by cystoscopy. This diagnostic review bias should be considered in the evaluation of (point of care) urine tests for bladder cancer monitoring.

Introduction

Looking inside the hollow organs and body cavities of human beings is a concept that was first put forward in 1806 by Philipp Bozzini. Already in 1877 Maximilian Carl-Friedrich Nitze (1848-1906) performed the first cystoscopy in Vienna (1). Since then, cystoscopy has developed as the mainstay in diagnosis of urinary bladder disease with a high sensitivity and specificity for the detection of papillary lesions (2-4). The availability of flexible cystoscopes further improved its acceptance as a diagnostic tool. Nevertheless, alternative non-invasive diagnostic methods are being investigated in order to reduce the cystoscopy frequency, because cystoscopy is considered an invasive, time-consuming and costly procedure and burdensome for the patient (5,6). Several authors suggested that implementation of urine tests, such as microsatellite analysis (MA) for detection of tumour cells in voided urine samples, may represent an acceptable alternative for diagnosis of both recurrent UC of the urinary bladder and detection of upper urinary tract UC (7-9).

For detection of flat lesions, like hyperplasia, dysplasia and carcinoma in situ white light cystoscopy has a lower sensitivity compared to papillary lesions. Nonetheless, hand, the sensitivity of cystoscopy for detection of papillary lesions is by definition very high since alternative diagnostic tests are not in routine use for detection of UC recurrence and – as a consequence – its alleged high sensitivity cannot be verified independently.

We embarked on a randomized multicenter study on the safety and the cost-effectiveness of surveillance of patients with low-grade (WHO 1973 grade 1 and 2) NMI UC using microsatellite analysis on voided urine samples as an alternative for cystoscopy(10). In the control arm of this study the urine test outcome was not communicated to the attending urologist during the follow-up visits, but for the patients randomized to the test arm the urologist in charge was aware of the urine test outcome. In this paper we investigated whether diagnostic review bias may have caused the observation that the number of recurrences was higher in the test arm than in the control arm.

Materials and Methods

Study design

A total of 484 patients with primary or recurrent NMI UC (i.e. TNM 1997 stage pTa, pT1, and WHO 1973 grade 1 or 2) were recruited by 10 Dutch hospitals for participation in the randomized CEFUB trial (Cost-Effectiveness of Follow-up of Bladder Cancer Trial). Of the 10 participating hospitals, three (one community and 2 academic centres) have an urologist residency-training program. The mean number of patients contributed by each hospital was 45 patients (range$^{8-128}$). White light cystoscopy was employed for surveillance by the 10 hospitals. All participating urologists were informed about the literature on the performance of MA for

the detection of recurrent UC (6,7). Patients with a history of carcinoma in situ or grade 3 UC were excluded from participation. After transurethral resection (TUR) of the bladder tumour, 448 were randomized after signing an informed consent form (registration: ClinicalTrials.gov NCT00126958, Supplemental Figure 7.3). The patients were stratified for hospital (ten hospitals), histopathological diagnosis (grade and stage) and *FGFR3* gene mutation status (mutation, wild type). Participants were assigned to two trial arms by block randomisation in order of appearance. Urologists of attendance received Case Record Forms (CRFs) with a unique trial number linked to a database in the centre of coordination. Study started at the first cystoscopy at 3 months after the TUR of inclusion with a follow-up either by 3-monthly cystoscopy alone (control arm) or by cystoscopy at 3 months, 12 months and 24 months and 3-monthly MA on voided urine samples (intervention arm). The urine test on voided urine samples, i.e. microsatellite analysis, was performed in both randomized groups as described in detail before (6,10). The laboratory analyses were performed without knowledge of clinical or histological status. Urine test results were only communicated (by mail) to the urologist of attendance in the test arm, and according to protocol a positive test was followed by a cystoscopy. The protocol did not prescribe (random) biopsies in the test arm in case of a positive urine test outcome. If (in the test arm) positive urine test outcome was incongruent with cystoscopy during two subsequent follow-up visits the protocol recommended imaging of the upper urinary tract. The primary outcome of the study was tumour recurrence, defined as the presence of a histopathologically confirmed UC detected by a cystoscopy during the follow-up. The ethical committees of the participating institutions approved the study.

Statistical Analysis

Data analysis of randomized groups was performed on an intention-to-treat basis, with log rank tests for Kaplan-Meier curves to compare the time to recurrence. Patients with a recurrence during follow-up remained in the same trial arm, with a similar follow-up scheme as after inclusion in the study. Subsequent recurrences were analyzed with modulated renewal (11). Recurrence rates per follow-up visit were compared using chi-square tests. The randomized study was originally powered to demonstrate equivalence between randomized groups, since follow-up of patients with microsatellite analysis on voided urine would be preferred in case of similar recurrence rates in the two arms. Equivalence was defined as that the arm with surveillance by urine tests was not more than 5% worse than the cystoscopy only arm, which implies that the 2-year recurrence risk is not less than 45% compared to 50%. Statistical power was set to 80% and the one-sided significance level set to 5%. Based on standard formulas for the required number of events in a log-rank test, 290 events should be registered during follow-up. To evaluate the influence of knowledge of the urine test outcome, chi-square tests would have 97% power to demonstrate differences in recurrence rates of 30% vs 10% with 50 recurrences among 250 follow-up visits with positive urine test results. Data were collected

using standardized CRFs and analyzed with SPSS 11.5 software (SPSS Inc., Chicago, Ill). P-values below 5% were considered statistically significant.

Results

Patients, tumours and urine samples

Patient recruitment was from July 2002 till June 2006 and follow-up ended by November 2006, with a median follow-up of 34 months. Patients in the intervention (unblinded for urine test outcome) (n=222) and control (blinded for urine test outcome; n=226) arms were comparable with regard to gender, pack years of smoking and age distribution, histopathology and disease status (primary versus recurrence) of their NMI UC at entry (Table 7.1). A total of 3,138 cystoscopies were performed (1501 in intervention arm, 1637 in control arm). A total of 1,398 voided urine samples were collected of which 1073/1398 (77%) samples were informative and in 23 % (325/1398) of urine samples the urine test failed due to various reasons (10). For 837 follow-up visits both cystoscopy and corresponding urine test results were available, i.e. for 391 and 446 follow-up visits in the intervention arm and control arm, respectively.

Detection of urinary bladder recurrences by cystoscopy in the two trial arms

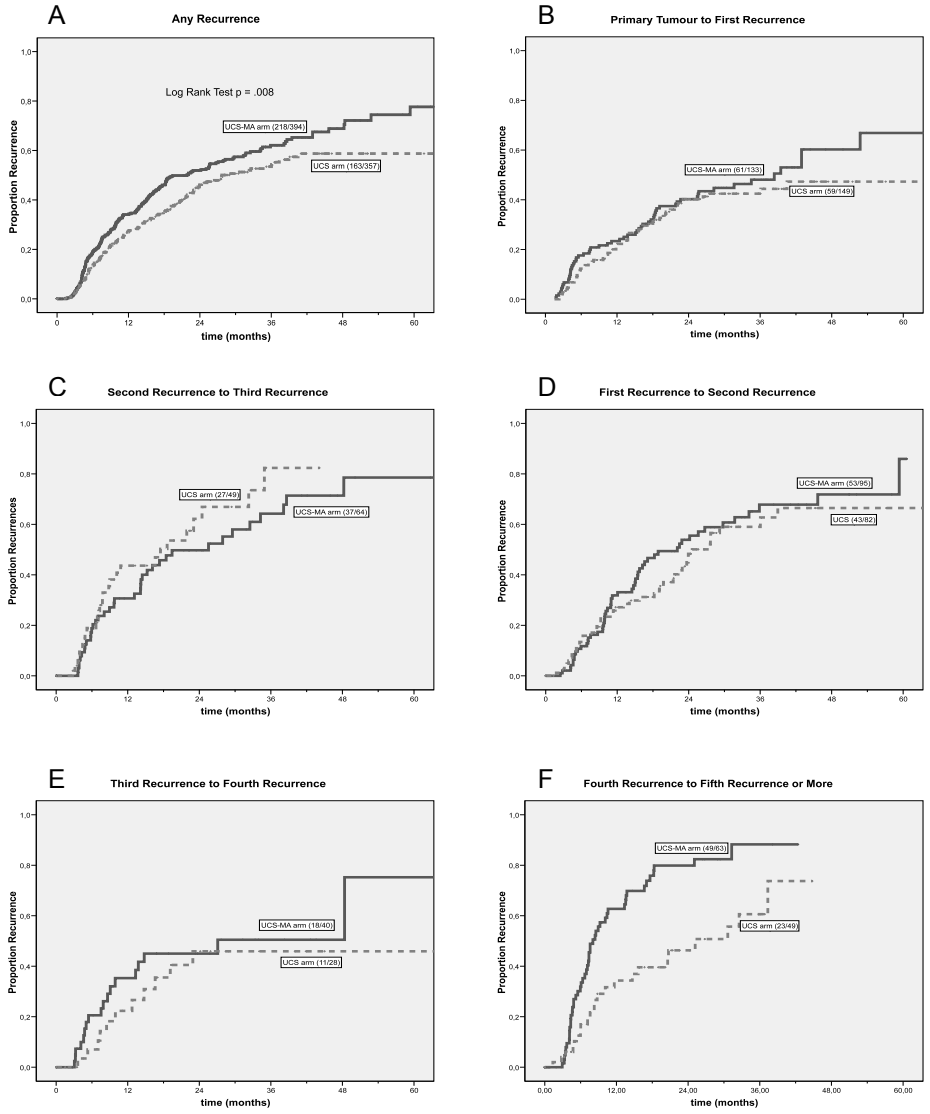
More recurrences were detected in the intervention arm as compared to control arm (log rank p=0.008, Figure 7.1A). Figure 7.1B-F shows the breakdown of the recurrences by rank of recurrence of each patient. In the intervention arm, 218 recurrences were detected at 1501 follow-up visits (14.5%), compared to 163 at 1637 visits (10.0%) in the control arm (chi-square test p<0.001, supplemental table 7.1). The false detection rates, that is the proportion of resected lesions *not* representing histologically confirmed carcinomas was slightly lower (i.e. 12%; 45/381) in the intervention arm than in the control arm (i.e. 16%; 52/319).

Table 7.1

Patient Characteristics (n=448)		intervention arm n = 222	control arm n = 226
Gender	Male	174	172
Age at study entry (years)	Mean ± sd	65 ± 12	66 ± 12
Smoking years	Mean ± sd	22 ± 18	25 ± 18
Histopathology	pTaG1	95	88
	pTaG2	88	95
	pT1G1	4	4
	pT1G2	35	39
Primary Tumor	number	133	149

Clinico-pathologic features of participants at entry of the trial

Figure 7.1 A - F



Kaplan Meier analyses of histologically proven UC recurrences during follow-up of all patients in the UCS-MA and UCS arm

Subset analysis of follow-up visits with synchronous cystoscopy and urine test results

A subset analysis was performed on the 391 follow-up visits in the intervention arm and 446 follow-up visits in the control arm, for which both cystoscopy and urine test results were available. Hence, we were able to find an explanation for the difference in the number of recurrences between the two trial arms. The number of follow-up visits without any intervention was lower in the intervention arm (81%; 317/391) as compared to the control arm (92%; 412/446, chi-square $p < 0.001$, Supplemental Table 7.2). A larger number of transurethral

Table 7.2

Urine test result	Trial arm	Number of follow-up visits	Number of recurrences (%)	P value*
positive	intervention	131	42 (32)	< .0001
	control	120	6 (5)	
negative	intervention	260	18 (7)	.45
	control	326	18 (6)	

* Pearson Chi-Square Test

Distribution of histologically confirmed UC recurrences according to MA outcome and trial arm detected in 837 follow-up moments for which both MA and cystoscopy results were available.

resections was performed after the follow-up visits in the intervention arm (n=61; 16%) than in the control arm (n=25; 6%, chi-square p < 0.001). Presence of a visible lesion did not lead to a resection in 21 out of 88 (24%) and in 14 out of 47 (30%) follow-up visits in the intervention and control arm, respectively (chi-square p=0.45).

Table 7.2 relates the number of histologically proven UC recurrences to cystoscopy results and to urine test outcome in both trial arms for those follow-up visits of which both cystoscopy and urine test outcome data were available. The proportion of histologically proven recurrences was significantly higher (p< 0.001) for the follow-up visits where a positive urine outcome was communicated to the urologist (intervention arm, 42/131, 32%) compared to the proportion of recurrences while the urologist was blinded for a positive urine test outcome (6/120, 5%). On the other hand, such a difference was not observed (p=0.45) for follow-up visits with a negative urine test outcome in the intervention arm (18/260, 7%) and control arm (18/326, 6%).

We also compared the performance of the urine test in both trial arms. Both if the analysis was done for all visits for which matched urine test and cystoscopy results were available (data not shown) or for those follow-up visits of the subset of patients whose cystoscopy was at the time of the MA test at the planned moments for cystoscopy (Table 7.3) the performance of the urine test in the intervention arm was much better than in the control arm.

Difference between hospitals with or without residence-training

The three hospitals with a urologist residency-training program (two academic and one community hospital) had a higher UC detection rate than the seven community centres without training program, i.e. 22% (271/1251) and 16% (332/2128), respectively (p<0.001). The seven community hospitals without a residency program had UC detection rates of 17% (195/1126) and 14% (137/1002) in the intervention and control-arms, while these numbers were 23% (141/616) and 21% (130/635) for the 3 hospitals with a residency training program, respectively.

Table 7.3

Comparison of accuracy of microsatellite analysis in the two trial arms

3A. Control arm; urine test results were blinded for urologists

Urine test outcome	UCC recurrence	No UCC recurrence	Total
Positive	0	53	53
Negative	10	164	174
Total	10	217	227

Sensitivity	0% (0/10)	95% CI [0.0-0.26]
Specificity	76% (164/217)	95% CI [0.69-0.81]
Positive Predictive Value	0% (0/53)	95% CI [0.0-0.06]
Negative Predictive Value	94% (164/174)	95% CI [0.89-0.97]

3B

Test arm; urine test results were communicated to urologists before cystoscopic examination

Urine test outcome	UCC recurrence	No UCC recurrence	Total
Positive	20	45	65
Negative	8	105	113
Total	28	150	178

Sensitivity	71% (20/28)	95% CI [0.51-0.87]
Specificity	70% (105/150)	95% CI [0.62-0.77]
Positive Predictive Value	31% (20/65)	95% CI [0.19-0.43]
Negative Predictive Value	93% (105/113)	95% CI [0.87-0.97]

Discussion

We found an increased number of recurrences by cystoscopy if information of a positive urine test was communicated to the urologist, but not if the result was kept blinded. If our analysis was restricted to the follow-up visits of the patients in which cystoscopy was planned next to the MA test, at the moments, we continued to discover more recurrences in the unblinded arm (Table 7.3). Hence, the increased detection rate in the unblinded study arm ('intervention arm') could not be attributed to ascertainment bias , but to diagnostic review bias (12). The positive urine test outcome may have changed the interpretation of the urologist of the findings during cystoscopy and/or increased the scrutiny of the cystoscopic examination resulting in the much higher number of histologically proven recurrences. In the blinded control arm no difference was observed between urine test positive (5%) and urine test negative (6%) follow-up visits.

The randomized design with a relatively large number of included patients allowed a reliable comparison of the number of recurrences detected in each trial arm. A limitation of our study is the modest compliance to the designed follow-up protocol, and informative urine samples were obtained only in 1073 / 3379 (32%) of the time a cystoscopy was performed.

Supplemental table 7.3

Interventions during 837 follow-up visits when MA outcome was available at time of cystoscopy in the UCS-MA (A) and UCS (B) arm

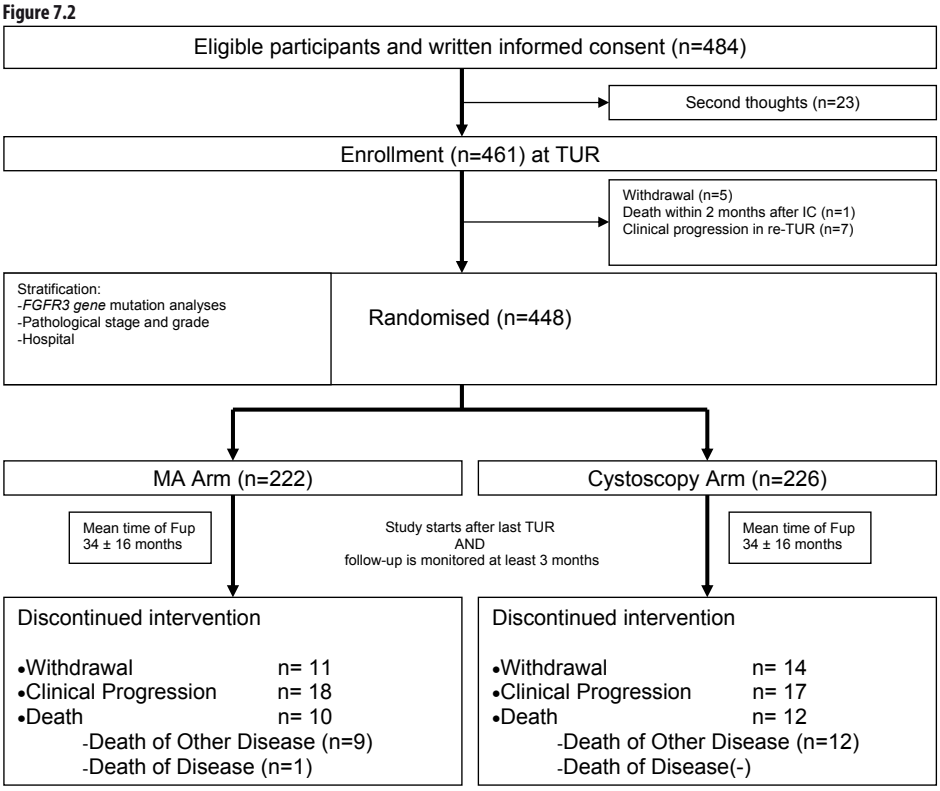
A. UCS-MA Group: MA results communicated to the urologist					
MA result	+	-	+	-	Total
Cystoscopy result	+	+	-	-	
Decision no treatment					
Continue Fup	0	0	75	221	296
Continue Fup despite a visible lesion in bladder	8	12	1	0	21
Treatment and additional diagnostics					
Outpatient clinic coagulation	1	2	0	0	3
Random Biopsy	2	4	0	0	6
Trans Urethral Resection (TUR)	39	19	1	2	61
Upper Urinary Tract lesion	1	0	3	0	4
Total number of follow-up visits	51	37	80	223	391

B. UCS-group: MA results NOT communicated to the urologist					
MA result	+	-	+	-	Total
Cystoscopy result	+	+	-	-	
No treatment					
Continue Fup	0	0	101	297	398
Continue Fup despite a visible lesion in bladder	11	3	0	0	14
Treatment and additional diagnostics					
Outpatient clinic coagulation	0	2	0	0	2
Random Biopsy	1	6	0	0	7
Trans Urethral Resection (TUR)	7	17	0	1	25
Total number of follow-up visits	19	28	101	298	446

The reduction in number of cystoscopies in the intervention arm as anticipated during the study design was not achieved and a lack of compliance by both patients and urologists may have contributed to this observation. Patients participating in the trial did not consider cystoscopy as very burdensome and they prefered to have an immediate result at the time of cystoscopy rather than to wait for a week for the outcome of the urine test (13)It is striking that the number of cystoscopically detected recurrences was also larger (n=33) during follow-up visits in the intervention arm when the synchronous urine test outcome was not available. Many of the patients with a recurrence had a positive urine test result antedating the follow-up visit at which the cystoscopy was performed (10) and knowledge of a positive urine test in the same patient, several months prior to the cystoscopy may also have influenced cystoscopy findings.

Importantly, the increased number of recurrences associated with the awareness of a positive urine test outcome was not associated with an increased false detection rate of recurrences by cystoscopy in the intervention arm. The false detection rate in both arms (12%,16%) is in the same range as previously reported for cystoscopy in other European series (13, 14).

Figure 7.2



Flow of participants through the CEFuB-trial (Douglas G. Altman et al, the revised CONSORT statement for reporting randomized trials: explanation and elaboration. Ann Intern Med. 2001; 134:663-694).

We also noted that the diagnostic review bias was somewhat more pronounced in the community hospitals without urologist-residency training program, implying a lower accuracy of the cystoscopy in these hospitals as compared to the three hospitals (including two academic hospitals) with a residence training program.

Several attempts are now being made to improve the sensitivity of cystoscopy for papillary and flat lesions of the urinary bladder by using photodynamic diagnosis. Photosensitizers proved their use in enhancing the visual demarcation between normal and neoplastic tissue (13-15). Therefore photodynamic cystoscopy has been used as a tool to assess the sensitivity of white light cystoscopy for the detection of papillary and flat lesions. In two studies, white light cystoscopy was performed first, followed by fluorescence cystoscopy on the same set of patients and both reported a sensitivity of white light cystoscopy and fluorescence-guided cystoscopy of respectively 83% and 95% (15, 16) for detection of papillary UC if analysed per lesion, and 97% and 100% respectively if analysed per patient (16). Our study points at much lower levels of sensitivity of cystoscopy, raising further doubt about the value of cystoscopy as the gold standard for UC recurrences. Probably, the threshold of urologists to decide whether a lesion observed during cystoscopy represents an urothelial neoplasm could be

lowered, triggering more frequent transurethral resections. Alternatively, ancillary detection techniques like our urine test may be of benefit to enhance the visibility and discrimination of urothelial neoplasms, providing a new gold standard for detection of these lesions.

Obviously, an important remaining question is whether the additional UC detected in the intervention arm represented clinically relevant tumours. A few papers have reported the lack of clinical impact of early detected recurrences raising the possibility that such lesions are amenable for expectant management (15,16). Comparison of the urinary bladder UC detected in both arms did not reveal differences in grade and stage contribution (data not shown). However, the only 3 carcinomas in situ detected by cystoscopy during follow-up were in the intervention arm. Further, fluorescence cystoscopy studies have shown that improved bladder cancer detection during surveillance may reduce long-term recurrence rates and costs of treatment (15).

We conclude that communication of a positive urine test outcome to the urologist is associated with a substantial diagnostic review bias. This will lead to an underestimation of the sensitivity of a urine test when the urologist is blinded for a positive test outcome while performing cystoscopy. As a consequence, the interpretation of the results of (newly developed) point of care urine tests should take this potentially strong bias into account. In addition, our resutls question the value of cystoscopy as the gold standard for detection of NMI UC recurrence.

ZON-MW, The Netherlands Organization for Health Research and Development, funded this study grant number 945-02-046. The funding source had no involvement in the trial.

References

1. Herr HW. Max Nitze, the cystoscope and urology. J Urol 2006;176(4 Pt 1):1313-6.
2. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. Pharmacoeconomics 2003;21:1315-30.
3. Wright MP, Jones DJ. Surveillance for bladder cancer: the management of 4.8 million people. South-west Urologists. BJU Int 2000;85:431-3.
4. Mariappan P, Smith G. A surveillance schedule for G1Ta bladder cancer allowing efficient use of check cystoscopy and safe discharge at 5 years based on a 25-year prospective database. J Urol 2005;173:1108-11.
5. Riley GF, Potosky AL, Lubitz JD, Kessler LG. Medicare payments from diagnosis to death for elderly cancer patients by stage at diagnosis. Med Care 1995;33:828-41.
6. van Rhijn BW, Lurkin I, Kirkels WJ, van der Kwast Th, Zwarthoff EC. Microsatellite analysis--DNA test in urine competes with cystoscopy in follow-up of superficial bladder carcinoma: a phase II trial. Cancer 2001;92:768-75.
7. Steiner G, Schoenberg MP, Linn JF, Mao L, Sidransky D. Detection of bladder cancer recurrence by microsatellite analysis of urine. Nat Med 1997;3:621-4.
8. Seripa D, Parrella P, Gallucci M, Gravina C, Papa S, Fortunato P, et al. Sensitive detection of transitional cell carcinoma of the bladder by microsatellite analysis of cells exfoliated in urine. Int J Cancer 2001;95:364-9.
9. Mao L, Schoenberg MP, Scicchitano M, Erozan YS, Merlo A, Schwab D, et al. Molecular detection of primary bladder cancer by microsatellite analysis. Science 1996;271(5249):659-62.
10. Van der Aa M, Zwarthoff EC, Steyerberg EW, Boogaard MW, Nijsen Y, van der Keur KA, van Exsel AJ, Kirkels WJ, Bangma C, van der Kwast Th. Microsatellite Analysis of voided urine samples for surveillance of low-grade non-muscle invasive urothelial carcinoma: feasibility and clinical utility in a prospective multicenter study (CEFUB). European Urology, 2008, in press.
11. Oakes D, Cui L. On Semiparametric Inference for Modulated Renewal Processes . Biometrika 1994; 81, 83-90]
12. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med 2003 138: W1-W12.
13. Van der Aa MN, Steyerberg EW, Sen EF, Zwarthoff EC, Kirkels WJ, van der Kwast Th, Essink-Bot ML. Patients' perceived burden of cystoscopic and urinary surveillance of bladder cancer: a randomized comparison. BJU Int. 2008 May;101(9):1106-10.
14. Schmidbauer J, Witjes F, Schmeller N, Donat R, Susani M, Marberger M. Improved detection of urothelial carcinoma in situ with hexaminolevulinate fluorescence cystoscopy. J Urol 2004; 171: 135-8
15. Jocham D, Witjes F, Wagner S, Zeylemaker B, Van Moorselaar J, Grimm MO, et al. Improved detection and treatment of bladder cancer using Hexvix imaging: a prospective phase III multi-center study. J Urol 2005; 174: 862-6.
16. Soloway MS, Bruck DS, Kim SS. Expectant management of small, recurrent, noninvasive papillary bladder tumors. J Urol. 2003;170(2 Pt 1):438-41.
17. Pruthi RS, Baldwin N, Bhalani V, Conservative management of low risk superficial bladder tumors. J Urol. 2008;179(1):87-90
18. Grossman HB, Gomella L, Fradet Y, Morales A, Presti J, Ritenour C, et al. A phase III, multicenter comparison of hexaminolevulinate fluorescence cystoscopy and white light cystoscopy for the detection of superficial papillary lesions in patients with bladder cancer. J Urol 2007;178:62-7.
19. Hungerhuber E, Stepp H, Kriegmair M, Stief C, Hofstetter A, Hartmann A, Knuechel R, Karl A, Tritschler S, Zaak D. Seven years' experience with 5-aminolevulinic acid in detection of transitional cell carcinoma of the bladder. Urology. 200; 69:260-4.

PART IV

THE PATIENT UNDER SURVEILLANCE

CHAPTER 8

Burden of cystoscopy

CHAPTER 9

Sexual function

CHAPTER 8

The burden of flexible cystoscopy in patients monitored for bladder cancer

Madelon N.M. van der Aa
Ewout W. Steyerberg
E. Fatma Sen
Ellen C. Zwarthoff
Wim J. Kirkels
Theo H. van der Kwast
Marie-Louise Essink-Bot

Abstract

Patients with non-muscle invasive low-grade (pTa / pT1, G1/G2) urothelial cell carcinoma of the urinary bladder are recommended to adhere to regular urethrocystoscopic surveillance (UCS). We assessed patients' perceived burden of flexible cystoscopy.

A total of 220 participants of a randomized trial comparing cystoscopic examination and surveillance by microsatellite analysis (MA) in voided urine were asked to fill out questionnaires one week after cystoscopy or urine sample collection. We assessed discomfort and pain as experienced during cystoscopy, experiences with MA and the waiting time for the result, and physical symptoms, medical consumption and general functioning. We analysed data from 732 questionnaires (197 patients) completed after cystoscopy and 184 questionnaires (67 patients) after collection of urine. Introduction of the cystoscope was reported to cause discomfort in 39% and pain in 35% of questionnaires. Collection of urine was not burdensome, but the waiting time for the results was reported as burdensome in 19%. Painful micturition was significantly more frequent in the week after cystoscopy than after MA (30% and 11%, respectively). The frequency of fever (1% and 2%) and haematuria (7% and 6%) was similar in both groups. Older patients reported significantly less pain and discomfort from cystoscopy, and this was not related to a larger number of previous cystoscopies. Flexible cystoscopy was quite burdensome in about one third of cases. The results of the present study are a further motivation in the search for less invasive surveillance tests.

Introduction

Bladder cancer is the fifth most common malignancy in Europe and the fourth most common malignancy in the United States. It affects one in 4000 people and accounts for 5% of all diagnosed cancers[1]. In 80% of the patients the bladder tumour will primarily appear as a papillary non-muscle invasive urothelial cell carcinoma (UC). The clinical course of non-muscle invasive (pTa and pT1) papillary UC is characterized by a high tendency to recur (up to 70%) and a propensity to progress in grade (10-30%) or stage (10-15%)[2, 3]. Therefore, patients are recommended to adhere to regular cystoscopic surveillance (UCS). The European Association of Urology (EAU) recommended 2 cystoscopies in the first year after diagnosis and then annual cystoscopies for up to five years in low-risk patients, but considerable practice variation is observed[4]. The effectiveness of cystoscopy on survival has been shown[5-8]. However, cystoscopy is an invasive procedure that may be associated with pain and discomfort. No studies systematically evaluated the impact of cystoscopy as perceived by patients[9-13]. The present study aimed at evaluation of the impact or burden of cystoscopy as perceived by patients under UCS in a randomized comparison with patients monitored by microsatellite analysis of urinary samples (MA).

Methods

Patients

This questionnaire study was part of a randomized clinical trial evaluating the cost-effectiveness of surveillance of patients with non-muscle invasive bladder cancer (CEFuB-trial) by microsatellite analysis (MA) of urinary samples for detection of cells with loss of heterozygosity in the DNA extracted from voided urine samples[14, 15] compared with conventional cystoscopic surveillance (UCS). The trial recruited patients with non-muscle invasive bladder cancer (pTa, pT1, G1/G2) from 14 centres in The Netherlands (2 teaching hospitals, 12 general hospitals) after informed consent. Patients were randomized into two groups: the UCS group, where patients had 3-monthly cystoscopy during 24 months; and the MA group, where patients had 3-monthly analysis of voided urine samples during 24 months. In the MA group, patients also had cystoscopy at 3, 12 and 24 months, and in case of a positive MA-test result. For the empirical evaluation of the burden of cystoscopy, a total of 220 patients from 10 centres from both trial arms were asked to fill out questionnaires at one week after cystoscopy or one week after collection of a urine sample for MA in the period between July 2003 and January 2006.

Ethics approval

The Medical Ethical Review Board of Erasmus MC – University Medical Center Rotterdam, The Netherlands, approved of the study.

Cystoscopy procedure

All patients underwent cystoscopy in lithotomy position with flexible cystoscopes for adults. Before introduction, the tip of the cystoscope was covered with an installation gel containing lidocaine. The result of the cystoscopy was communicated to the patient directly after the procedure.

MA procedure

Patients collected a urine sample in a vial during a scheduled visit at the outpatient clinic. The result of the MA was communicated to the patient by the urologist two weeks after delivery of the urine sample.

Questionnaires

Discomfort and pain during cystoscopy (Cystoscopy questionnaire)

Discomfort and pain were assessed by separate series of 4 items each for the 4 stages of the procedure: the preparation for the cystoscopy, the introduction of the cystoscope, undergoing the cystoscopy itself, and the hours directly after cystoscopy. Subjects were offered three response options ('not', 'quite' and 'very' painful or discomforting, respectively). Items were adapted from earlier studies[16-18].

Discomfort during MA procedure (MA questionnaire)

We assessed patients' experienced discomfort with urine collection, delivery at the outpatient clinic and the waiting period for the test result using similar items with three response options ('not', 'quite' or 'very' discomforting).

Physical symptoms and medical consumption in the week after cystoscopy / delivery of urine sample (both questionnaires)

We assessed occurrence and duration of painful micturition, urge and frequency symptoms and haematuria in the first week after cystoscopy / urine delivery with items with 3 response options ('no', 'yes, < 7 days', 'yes, > 7 days'). The occurrence of fever (> 38 degrees Celsius) was asked with a yes / no response format; if 'yes', patients were requested to enter the number of days. The need for medication (oral analgesics and antibiotics), and whether a general practitioner had been consulted for problems related to the cystoscopy or bladder problems (MA group) in the subsequent week were asked with a yes/no response format.

Impact on general functioning (both questionnaires)

Patients' general functioning in the week after cystoscopy or urine delivery for MA was assessed as the perceived impact of the cystoscopy or MA procedure, respectively, on daily

activities and social life in the week afterwards using two separate items with five response options ('not at all', 'little', 'quite', 'lots' and 'all the time'.)

Patient satisfaction (both questionnaires)

Satisfaction regarding the reception at the outpatient clinic, waiting time at the outpatient clinic, explanation of the procedures were assessed with 3 items with 3 response options ('not', 'quite' and 'very' satisfied). Items were based on previous work[19].

Determinants of perceived pain and discomfort, physical symptoms, general functioning and satisfaction(both questionnaires)

Data on age, gender, type of hospital (teaching or general), number of previous cystoscopies in an outpatient setting (cystoscopy experience), and number of previous Trans Urethral Resections (TURs) of non-muscle invasive bladder cancer (procedures requiring hospitalisation) were obtained from the case record forms of the CEFuB-trial.

Statistics

We analysed pain, discomfort, overall burden, physical symptoms, medical consumption, general functioning and satisfaction after cystoscopy or urine delivery for MA using all data from all questionnaires that were completed. The correlation between assessments from the same person was taken into account by using formal multilevel analysis (ProcMixed with REML, SAS version 9.1, SAS Institute Inc, Cary NC).

We estimated overall burden of either cystoscopy or MA by averaging item scores for pain and discomfort as experienced during cystoscopy, or the MA procedure, respectively. The averaged overall burden of cystoscopy consisted of the items on pain and discomfort related to introduction of the cystoscope, undergoing the cystoscopy and the period immediately afterwards (Cronbach's alpha = 0.88; scoring range 1 (no burden) - 3 (much burden)). The average overall burden of the MA procedure consisted of the sum of the ratings of discomfort experienced during collection of the urine sample, delivering the sample at the outpatient clinic, and the waiting for the test result.

We analysed the effects of determinants (age, gender, number of previous cystoscopies, number of previous TURs, and type of hospital on overall burden, physical symptoms (average score of 4 items on painful micturition, urge, fever and haematuria), medical consumption (average score of 3 items on pain medication or antibiotics and general practitioner visits), general functioning (average score of 2 items on daily and social functioning) and satisfaction (average score of 3 items for reception, waiting time and explanation) in univariate analyses using Spearman's ρ (using SPSS release 11.0.1 (SPSS Inc., Chicago, IL). We corrected for dependency in the data by using the ProcMixed procedure of SAS. P-values < 0.05 were considered statistically significant. Additionally, we analysed correlations between the determinants and a number of specific separate items.

Results

Response

A total of 201 out of 220 patients (92%) filled out at least one questionnaire. Of these 201 respondents, 108 had been randomized into the UCS-arm and 93 into the MA-arm. Subjects in the MA-arm underwent either MA analysis or cystoscopy at different moments during follow-up, and 62 of 93 subjects from the MA arm completed questionnaires both after cystoscopy and after MA. A total of 916 questionnaires were available for analysis: 732 questionnaires filled out by 197 patients after cystoscopy (mean 3.6 questionnaires per patient, range 1 to 8) and 184 questionnaires filled out by 67 patients after collection of urine (mean 2.8 questionnaires per patient, range 1 to 5). The characteristics of patients in both arms were very similar (Table 8.1).

Discomfort and pain as experienced during the procedure

Table 8.2 shows the percentages of reported discomfort and pain as experienced during the subsequent stages of cystoscopy and the MA procedure. The introduction of the cystoscope was considered most frequently as burdensome, being at least 'quite' discomforting in

Table 8.1
Characteristics of 201 patients with papillary urothelial cell carcinoma included in the CEFuB trial and participating in the questionnaire study.

		UCS	MA
Response		99% (108/110)	85% (93/110)
Gender	Male	73% (79/108)	77% (72/93)
Previous cystoscopies	1	15% (16/108)	22% (20/93)
	2-5	41% (45/108)	34% (32/93)
	> 5	44% (47/108)	44% (41/93)
Previous TURs	1	67% (72/108)	50% (47/93)
	2-5	23% (25/108)	38% (35/93)
	>5	10% (11/108)	12% (11/93)
Age (years)	Mean (SD)	68.3 (13.0)	68.2 (11.5)

UCS = urethrocystoscopic surveillance group
MA = microsatellite analysis (in voided urine) group

273/697 (39%) and at least 'quite' painful in 240/695 (35%) of the questionnaires. Spearman correlations between pain and discomfort scores for the same stage of cystoscopy were 0.7 or higher, indicating that many subjects who reported discomfort for e.g., introduction of the scope, also reported this stage of cystoscopy to be burdensome.

Table 8.2
Frequency of pain and discomfort experienced during cystoscopy and physical symptoms in the week after cystoscopy (data from 732 questionnaires)

		No	Quite	Very	n
Discomfort	Preparation	614 (85.9%)	89 (12.4%)	12 (1.7%)	715
	Introduction	424 (60.8%)	229 (32.9%)	44 (6.3%)	697
	Undergoing	472 (67.9%)	193 (27.8%)	30 (4.3%)	695
	Hours after cystoscopy	517 (73.8%)	151 (21.5%)	33 (4.7%)	701
Pain	Preparation	673 (94.0%)	41 (5.7%)	2 (0.3%)	716
	Introduction	455 (65.5%)	215 (30.9)%	25 (3.6%)	695
	Undergoing	544 (77.9%)	135 (19.3%)	19 (2.7%)	698
	Hours after cystoscopy	535 (76.2%)	152 (21.7%)	15 (2.1%)	702
1 week after cystoscopy		No	Yes, < 7 days	Yes, > 7 days	
Painful micturition		500 (69.5%)	206 (28.7%)	13 (1.8%)	719
Urge and frequency		472 (66.0%)	194 (27.1%)	49 (6.9%)	715
Fever > 38C		709 (98.7%)	9 (1.3%)	0	718
		No	Yes, some	Yes, a lot	
Haematuria		664 (92.5%)	47 (6.5%)	7 (1.0%)	718

For MA, the period of waiting for the results of the urinary test was reported as at least 'quite' discomforting in 31/161 (19%) questionnaires. As expected, urine collection and delivery were not associated with discomfort.

Averaged overall burden of cystoscopy was 1.33 (standard error 0.017), of MA 1.09 (standard error 0.017), p-value of the difference <0.001. Among 48 patients for whom we had complete data on pain and discomfort after a cystoscopy and of an MA procedure, averaged overall burden of cystoscopy was 1.37 and of MA 1.09 (p<0.001, paired sample t-test).

Physical symptoms in the first week after cystoscopy / urine sampling

Painful micturition was reported in 219/719 (30%) of the questionnaires after cystoscopy and in 21/176 (12%) of the questionnaires after collection of urine (p<0.001). Symptoms of urge and frequency were reported in 243/715 (34%) and 45/177 (25%) of the questionnaires after cystoscopy and MA, respectively (p=0.09, Table 8.2). Haematuria and fever occurred infrequently (Tables 8.2 and 8.3).

Medical consumption in the week after cystoscopy / urine collection

Use of pain medication or antibiotics after cystoscopy was reported in 23/714 (3%) and 69/719 (10%) of the questionnaires, respectively, and in 9/175 (5%) and 18/177 (10%) of the questionnaires completed in the MA procedure. The general practitioner was consulted

Table 8.3
Frequency of discomfort and physical symptoms in the week after delivery of urine for MA analysis (data from 184 questionnaires)

		No	Quite	Very	n
Discomfort	Collection	158 (98.1%)	2 (1.3%)	1 (0.6%)	161
	Delivery	168 (97.6%)	4 (2.4%)	0	172
	Waiting for result	130 (80.7%)	24 (14.9%)	7 (4.3%)	161
1 week after urine collection		No	Yes, < 7 days	Yes, > 7 days	
Painful micturition		155 (88.1%)	19 (10.8%)	2 (1.1%)	176
Urge and frequency		132 (74.6%)	30 (16.9%)	15 (8.5%)	177
Fever > 38C		175 (98.3%)	3 (1.7%)		178
		No	yes, some	yes, a lot	
Haematuria		166 (93.3%)	10 (5.6%)	2 (1.1%)	178

infrequently in the week after either procedure (8/721 after cystoscopy (1%) and 3/177 in the MA group (2%)).

General Functioning

After cystoscopy, at least 'a little' impact on daily activities was reported in 134/720 (19%) of questionnaires, and at least 'a little' impact on social activities was reported in 86/723 (12%). Similar results were reported after MA: 33/175 (19%) reported at least 'a little' impact of the MA procedure on daily activities and 25/173 (14%) on social activities.

Satisfaction

Dissatisfaction with the reception at the outpatient clinic, the waiting time for cystoscopy, and the explanations by the staff was reported in 13/718 (2%), 51/709 (7%) and 25/710 (4%) of the questionnaires completed after cystoscopy. For MA these figures were 12/157 (8%), 13/173 (8%) and 20/167 (12%), respectively.

Determinants of burden of cystoscopy or MA

Averaged overall burden of cystoscopy (ρ=-0.16, $p<0.001$) and of MA (ρ=-.23, $p=0.024$) correlated only significantly with age. This significant correlation of overall burden of MA was attributable to the waiting time for the result (ρ=-0.17, $p=0.03$)

The correlation of overall burden of cystoscopy with the other determinants, including the number of previous cystoscopies and gender, were non-significant. The significant correlation of reported burden with age was not attributable to a larger number of previous cystoscopies. Regarding specific aspects of the cystoscopy, men did not report significantly more burden from either introducing or undergoing the cystoscopy than women.

Physical symptoms, medical consumption and general functioning in the week afterwards were not correlated with any of the determinants investigated. Satisfaction correlated significantly with age (ρ=0.19, $p<0.001$).

Increasing age was associated with less reported overall burden of cystoscopy, and less discomfort from the waiting time for the result of MA analysis. Older subjects were more satisfied with the procedure. The correlations were however rather low.

Discussion

We analysed the burden of cystoscopy as perceived by patients under regular surveillance for non-muscle invasive UC during the different stages of cystoscopy and in the subsequent week. We found that cystoscopy was reported to be associated with pain and discomfort in about one-third of the questionnaires. In comparison with patients who underwent MA analysis of voided urine as surveillance test, the average overall burden reported after cystoscopy was significantly higher that after MA. The MA group was considered an appropriate control group as they shared the same underlying disease and the randomized design precluded selection bias. Burden of MA appeared fully attributable to the waiting time for the test result.

The burden of cystoscopy was most related to the introduction of the cystoscope, which was frequently reported to cause pain and discomfort. Herr et al reported an average pain score of 2 on a 10-point visual analogue scale ranging from 1 (no pain) to 10 (most pain) for men with non-muscle invasive bladder cancer who underwent flexible cystoscopy[13]. In our study, men did not report more pain and discomfort in association with the introduction of the cystoscope than women. In the study by Vriesema et al 56/85 (66%) of patients reported urethrocystoscopy to be bothersome, which was also similar for male and female patients[9]. We found a weak but significant inverse association of overall burden of cystoscopy and age, while Vriesema found no such association[9].

Cystoscopy is an invasive procedure, but the test result is immediately available to the patient. With MA analysis patients have to wait 10 – 14 days for the test result, and if it is positive, they are referred for cystoscopy in second instance. Vriesema et al[9] showed that patients may not easily exchange cystoscopic surveillance for a less sensitive method for detection of recurrences, even if the latter is associated with much less discomfort. In their study, patients were offered two alternative surveillance tests (cystoscopy and a hypothetical test based on urinary analysis). If the sensitivity of the urinary test fell below 90%, 89% of the patients would prefer flexible cystoscopy for surveillance of non-muscle invasive bladder cancer. Thus, patients seem to choose for the safe side. If there is a recurrence, they want their surveillance test to detect it, even if it means that they have to undergo an invasive surveillance test.

Micturition was reported to be painful in the week after cystoscopy in about 30% of the questionnaires, suggesting an effect of the cystoscopy. However, the 12% prevalence of painful

micturition in the MA group was unexpectedly high and goes sofar unexplained. Non-muscle invasive bladder cancer is not associated with irritative voiding symptoms. Symptoms of urge and frequency of voiding were reported to be about equally prevalent in the week after cystoscopy and delivery of a urine sample for MA. Fever and haematuria were not reported more frequently after cystoscopy than after MA.

In the comparison of our results with the few other reported empirical studies on the burden of cystoscopy, the association of symptoms after cystoscopy with the underlying disease must be kept in mind. Kortmann evaluated 103 men with lower urinary tract symptoms undergoing urodynamic evaluation with or without flexible cystoscopy[11]. Fifty-six % of the men in this study reported urge symptoms after urodynamic evaluation combined with cystoscopy. After urodynamic evaluation alone, 29% reported voiding discomfort, after urodynamic evaluation + cystoscopy this was 35%.

Denholm compared flexible and rigid cystoscopy in the early years after the introduction of the technique of the flexible cystoscopy. In this study, 89% of patients who underwent flexible cystoscopy for various reasons reported it to be painless, and 33% of them had physical symptoms in the week afterwards[12].

We showed further that surveillance by itself had an impact on patients, regardless of the surveillance test. Similar proportions of patients from both arms reported an impact on daily life and social life, irrespective of having undergone cystoscopy or MA. This seems to confirm the findings from the study reported by Yoshimura, who found that Japanese patients with non-muscle invasive bladder cancer had significantly impaired general health perceptions and mental health. However, mental health appeared to return to normal in patients with a longer history of disease. Yoshimuri et al concluded that, despite the favourable prognosis of non-muscle invasive bladder cancer, urologists should be aware of the considerable effect on patients' general health-related quality of life[20].

Our study has some limitations. The general functioning scores of our study may be affected by the timing of the assessments: at the time of completion, the results of cystoscopy were known to the subjects, whereas the MA result was still to be expected. The bother of the test itself is only one part of the burden of surveillance. The present report does not elaborate on the burden caused by being a bladder cancer patient and awareness of the prognosis of bladder cancer.

Conclusion

The clinician must be aware of the burden that is caused by cystoscopy of patients with non-muscle invasive bladder cancer. Patients will adhere to cystoscopic surveillance as long as cystoscopy is considered the golden standard; however, the test itself was reported to cause pain and discomfort in one third of the cases. We need to find alternative surveillance tests, diagnostically as good as or better than this invasive and truly burdensome procedure. The present study underscores and emphasizes the need for new less invasive methods for surveillance and hopefully encourages both scientists as urologists to continue this search.

References

1. Sengupta N, Siddiqui E, Mumtaz FH: Cancers of the bladder. J R Soc Health 2004, 124(5):228-229.
2. Allard P, Bernard P, Fradet Y, Tetu B: The early clinical course of primary Ta and T1 bladder cancer: a proposed prognostic index. Br J Urol 1998, 81(5):692-698.
3. Kurth KH, Denis L, Bouffioux C, Sylvester R, Debruyne FM, Pavone-Macaluso M, Oosterlinck W: Factors affecting recurrence and progression in superficial bladder tumours. Eur J Cancer 1995, 31A(11):1840-1846.
4. Witjes JA, Melissen DO, Kiemeney LA: Current practice in the management of superficial bladder cancer in the Netherlands and Belgian Flanders: a survey. Eur Urol 2006, 49(3):478-484.
5. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R: The health economics of bladder cancer: a comprehensive review of the published literature. Pharmacoeconomics 2003, 21(18):1315-1330.
6. Wright MP, Jones DJ: Surveillance for bladder cancer: the management of 4.8 million people. South-west Urologists. BJU Int 2000, 85(4):431-433.
7. Mariappan P, Smith G: A surveillance schedule for G1Ta bladder cancer allowing efficient use of check cystoscopy and safe discharge at 5 years based on a 25-year prospective database. J Urol 2005, 173(4):1108-1111.
8. Riley GF, Potosky AL, Lubitz JD, Kessler LG: Medicare payments from diagnosis to death for elderly cancer patients by stage at diagnosis. Med Care 1995, 33(8):828-841.
9. Vriesema JL, Poucki MH, Kiemeney LA, Witjes JA: Patient opinion of urinary tests versus flexible urethrocystoscopy in follow-up examination for superficial bladder cancer: a utility analysis. Urology 2000, 56(5):793-797.
10. Muezzinoglu T, Ceylan Y, Temeltas G, Lekili M, Buyuksu C: Evaluation of pain caused by urethrocystoscopy in patients with superficial bladder cancer: a perspective of quality of life. Onkologie 2005, 28(5):260-264.
11. Kortmann BB, Sonke GS, D'Ancona F C, Floratos DL, Debruyne FM, De La Rosette JJ: The tolerability of urodynamic studies and flexible cysto-urethroscopy used in the assessment of men with lower urinary tract symptoms. BJU Int 1999, 84(4):449-453.
12. Denholm SW, Conn IG, Newsam JE, Chisholm GD: Morbidity following cystoscopy: comparison of flexible and rigid techniques. Br J Urol 1990, 66(2):152-154.
13. Herr HW, Schneider M: Outpatient flexible cystoscopy in men: a randomized study of patient tolerance. J Urol 2001, 165(6 Pt 1):1971-1972.
14. Mao L, Schoenberg MP, Scicchitano M, Erozan YS, Merlo A, Schwab D, Sidransky D: Molecular detection of primary bladder cancer by microsatellite analysis. Science 1996, 271(5249):659-662.
15. van Rhijn BW, Lurkin I, Kirkels WJ, van der Kwast Th, Zwarthoff EC: Microsatellite analysis--DNA test in urine competes with cystoscopy in follow-up of superficial bladder carcinoma: a phase II trial. Cancer 2001, 92(4):768-775.
16. Kruijshaar ME, Kerkhof M, Siersema PD, Steyerberg EW, Homs MY, Essink-Bot ML: The burden of upper gastrointestinal endoscopy in patients with Barrett's esophagus. Endoscopy 2006, 38(9):873-878.
17. Essink-Bot ML, de Koning HJ, Nijs HG, Kirkels WJ, van der Maas PJ, Schroder FH: Short-term effects of population-based screening for prostate cancer on health-related quality of life. J Natl Cancer Inst 1998, 90(12):925-931.
18. Essink-Bot ML, Rijnsburger AJ, van Dooren S, de Koning HJ, Seynaeve C: Women's acceptance of MRI in breast cancer surveillance because of a familial or genetic predisposition. Breast 2006, 15(5):673-676.
19. Nijs HG, Essink-Bot ML, DeKoning HJ, Kirkels WJ, Schroder FH: Why do men refuse or attend population-based screening for prostate cancer? J Public Health Med 2000, 22(3):312-316.
20. Yoshimura K, Utsunomiya N, Ichioka K, Matsui Y, Terai A, Arai Y: Impact of superficial bladder cancer and transurethral resection on general health-related quality of life: an SF-36 survey. Urology 2005, 65(2):290-294.

CHAPTER 9

Sexual function of patients under surveillance for bladder cancer

Madelon NM van der Aa
Milou D Bekker
Fatma EF Sen
Theo H van der Kwast
Marie-Louise Essink-Bot
Ewout W Steyerberg
Ellen C Zwarthoff
Henk W Elzevier

ZON-MW, The Netherlands Organization for Health Research and Development, funded this study grant number 945-02-046, in collaboration with the Pelvic Floor & Sexuality Research group Leiden.

Abstract

Patients just diagnosed with non-muscle invasive bladder cancer (NMI UC) have the prospect of an intensive surveillance scheme by urethrocystoscopy (UCS) in order to detect tumour recurrences. Sexual dysfunction associated with this urological condition is commonly overlooked. We aimed to describe the prevalence of sexual dysfunction in this patient population and evaluated risk factors. We performed a cross-sectional survey on 150 patients just diagnosed with primary or recurrent NMI UC. Patients were participating in a randomized clinical multicenter trial (CEFuB), comparing two surveillance schemes. Patients were asked to fill in questionnaires at study entry 3 months before the start of the study-surveillance scheme (Demographic characteristics, validated Visual Analog Scale and validated subset of questions concerning sexual function and performance derived from QLQ-BLS-24). Results were compared to the age-matched healthy population. The response rate was 95% (142/150). A total of 61% (87/142) of the respondents was sexually active in the past four weeks after diagnosis, 66% of (70/105) males and 46% (17/37) of females. Although libido was not negatively affected, 54% (47/87) of the patients experienced sexual dysfunction, 23% (17/73) was afraid to inflict harm to their partner by sexual contact. Sexually active patients perceive a higher state of general health (p=.03). The prevalence of sexual dysfunction in patients with NMI UC is very high (54%) compared to an age and gender matched healthy population (20-45%). No predictors for sexual dysfunction were found. These patients and partners would benefit from proper sexual information in the outpatient clinic.

Introduction

Bladder cancer is the fifth most common malignancy in Europe and the fourth most common malignancy in the United States. It affects one in 4000 people and accounts for 5% of all diagnosed cancers[1]. In 80% of the patients the bladder tumour will primarily appear as a papillary non-muscle invasive (stage pTa and pT1) urothelial carcinoma (NMI UC). Up to 70% of these tumours will recur and 15% will progress in stage or grade[2]. Therefore, patients are recommended to adhere to regular cystoscopic surveillance (UCS). Besides patients with recurrent disease experience repeated transurethral resections and intravesical chemo / immunotherapy may be required to treat recurrences. Although the effectiveness of cystoscopy on survival has been shown[3-6], several studies have proven that cystoscopy is an invasive procedure that is perceived as burdensome and is associated with pain and discomfort[6-12]. Research of the impact on sexual function in patients with bladder cancer is limited and mostly focused on the treatment modalities of muscle invasive UC by cystectomy. Allareddy reported an adverse effect in sexual function after cystectomy in patients with muscle invasive bladder cancer[13]. However, less is known of the sexual function in patients under surveillance with non-muscle invasive UC. A study by Stav et al showed that rigid cystoscopy, performed on males and females, transiently impairs functional sexual performance and libido in sexually active patients[14]. The present cross-sectional questionnaire study aimed to describe the sexual function in patients who are just diagnosed with primary or recurrent NMI UC and face a 3-monthly intensive surveillance scheme. Prevalence of sexual dysfunction and sexual activity was compared to age and gender matched healthy populations. Furthermore we aimed to identify risk factors for sexual dysfunction.

Patients and Methods

Patients

We performed a cross-sectional questionnaire survey of sexual function in patients just diagnosed with primary or recurrent NMI UC prior to initiation of a surveillance protocol. This questionnaire study was part of a randomized controlled clinical trial (registration: ClinicalTrials.gov NCT00126958) evaluating the cost-effectiveness of surveillance of patients with NMI UC (CEFUB trial). A total of 448 patients with NMI UC (i.e. TNM 1997 stage pTa, pT1, and WHO 1973 grade 1 or 2) were recruited by 10 Dutch hospitals for participation after signing an informed consent form. In this questionnaire study we approached a subset of 150 patients in 7 hospitals who participate in the CEFUB trial and included patients randomly assigned by appearance in a period from July 2004 till September 2006. Patients were asked to fill in questionnaires at start of surveillance less than 3 months after diagnosis of primary or recurrent NMI UC. Patients in both randomized groups were monitored and treated equally at this

point. Data on age, gender, history of recurrent UC and intra-vesical therapy were obtained from the case record forms of the CEFUB trial.

Ethics Approval

The Medical Ethical Review Board of Erasmus MC - University Medical Center Rotterdam, and Leids University Medical Center, Leiden, The Netherlands, approved of the study.

Cystoscopy Procedure

All patients were monitored with surveillance by cystoscopy in lithotomy position. Flexible 17 Charier cystoscopes were used for male patients and rigid 20 Charier scopes for female patients. Before introduction, male urethra was instilled with 20cc lidocaine containing jelly or the tip of the rigid scope was covered with the lidocaine containing jelly. The result of the cystoscopy was communicated to the patient directly after the procedure.

Questionnaires

Visual Analog Scale (VAS)

We assessed patients' opinion about their general state of health by using a vertical positioned graduated ruler scaled from 0-100% (a validated generic health-related quality of life (HRQOL) instrument). Patients in both groups were asked to draw a line from a point left of the ruler to the experienced percentage of their present state of health.

Sexual function

We assessed patients' sexual life using a validated subset of 8 questions concerning sexual performance (erectile and ejaculatory function in male, level of lubrication in females) and function and libido (interest, enjoyment, level of activity, fear harming the partner) extracted from the EORTC QLQ-BLS 24 questionnaire. Subjects were offered four response options ('not at all', 'rather', 'a little' and 'very'). The appendix lists the different questions.

Statistics

We analysed all data using SPSS release 11.5 (SPSS Inc., Chicago, Ill). We analysed patients' assigned health state and sexual function using all data from all questionnaires that were completed. Differences in sexual activity (yes/no) between gender and smoking behaviour were analysed by Chi-square Test, between Age and score on the VAS by Students T Test and between the demographic variables by One-way ANOVA Test. Relation between various clinical characteristics and the fear to inflict harm to their partner by sexual contact is analysed by Students T Test (age, VAS, number of recurrences) and by Chi-square Test (gender, intravesical therapy (yes/no), history (just diagnosed with a primary tumour / with recurrent tumour), sexual performance and libido). P-values < 0.05 were considered statistically significant.

Results

A total of 142 out of 150 patients (95%) completed the questions concerning sexual contact in the past four weeks. 61% (87/142) of the respondents was sexually active in the past four weeks. Table 9.1 shows the demographic characteristics related to the sexual activity. Males showed significantly more sexual activity than females (chi square test; p=.02). Aging has a negative effect on sexual activity (independent students T-test; p=.02). Noticeable is the significantly higher perception of general health state in patients who are sexually active (independent students T-test; p=.03). Reasons for sexual inactivity were related to the single status, disablement and aging. Table 9.2 lists the perceived sexual function in the 87 sexual active patients according to 70 males and 17 females. Although the libido (interest in sexual contact) of the majority of the respondents was not scored as negatively affected, 54% (47/87) of the patients experienced some degree of erectile dysfunction or problems with

Table 9.1
Demographic characteristics of 142 patients with diagnosis of NMI UC concerning sexual activity

Characteristics of 142 patients		Sexual Non-active (n=55)	Sexual Active (n=87)	p value
Gender	Male	35 (33%)	70 (67%)	
	Female	20 (54%)	17 (46%)	.02
Age	Mean ± sd in years	69 ± 12	65 ± 10	
	Median [range] in years	72 [35-89]	66 [37-83]	.02
Smoke	Yes	45 (38%)	75 (62%)	
	No	9 (47%)	10 (53%)	.4
Education	Vocational Education	14 (45%)	17 (55%)	
	Secondary school	17 (39%)	27 (61%)	
	Advanced secondary school	7 (41%)	10 (59%)	
	Higher Vocational Education	10 (29%)	24 (71%)	
	University	6 (40%)	9 (60%)	.3
Marital Status	Married / Cohabitation	37 (33%)	75 (67%)	
	Single	4 (80%)	1 (20%)	
	Divorced	5 (42%)	7 (58%)	
	Widow (-er)	7(78%)	2 (22%)	.1
Current domestic situation	Together	34 (33%)	68 (67%)	
	Together + kids	4 (29%)	10 (71%)	
	Single	15 (68%)	7 (32%)	.006
Profession	Steady job	10 (25%)	30 (75%)	
	Voluntary job	6 (46%)	7 (54%)	
	Disablement Insurance Benefit	7 (78%)	2 (22%)	
	Retirement	32 (40%)	48 (60%)	.5
VAS	Mean ± sd	78 ± 14	84 ± 12	.03

Table 9.2

Sexual function in 87 sexually active males and females with NMI UC

Sexual Function		Female (n=17)	Male (n=70)	Total (%)
Interest in sexual contact	Yes	15	62	89%
	No	2	7	11%
Erectile or Vaginal* Dysfunction	Yes	7	40	55%
	No	9	29	45%
Trouble ejaculation	Yes		25	35%
	No		44	65%
Uncomfortable thinking of sexual contact	Yes	2	10	17%
	No	14	46	83%
Afraid to harm your partner	Yes	3	14	24%
	No	13	43	76%
Enjoyed sex	Yes	15	56	93%
	No	1	4	7%

* non-lubricating vagina

Table 9.3

Characteristics of 73 sexually active patients related to the fear to inflict harm to their partner by sexual contact

Afraid to harm their partner by sexual contact		Not afraid (n=56)	Afraid (n=17)	p value
Clinical Characteristics				
Age in years	Mean ± sd	64 ± 11	63 ± 7	.6
VAS in %	Mean ± sd	84 ± 13	86 ± 10	.5
Number of recurrences	Mean ± sd	1.7 ± 1.5	1.7 ± 1.1	.9
Gender	Male	43	14	
	Female	13	3	.6
IV	No	45	17	
	Yes	11	0	.04
History	Primary	40	12	
	Recurrent	16	5	.9
Sexual performance and libido				
Interest	Yes	56	12	
	No	0	5	< .000
Uncomfortable with thought of sexual contact	Yes	7	5	
	No	49	12	.07
Erectile or Vaginal* Dysfunction	Yes	26	10	
	No	30	7	.5

* non-lubricating vagina

vaginal lubrication. Thereby 23% (17/73) of the sexually active patients answered to be afraid to inflict harm to their partner by sexual contact; 85 patients answered this latter question, 12 were sexually inactive (9 males, 3 females). We found that three of the sexual inactive patients (3/12, 25%) were afraid to harm their partner; all three were males and reported to be sexually interested (Table 9.3). Questionnaires revealed that 12/73 (16%) patients were uncomfortable with the thought of sexual contact, in 42% (5/12) was this thought related to the fear to harm their partner in sexual contact (p= .07).

Discussion

The present cross-sectional questionnaire study aimed to describe the prevalence of sexual dysfunction and its components associated with the diagnosis of primary or recurrent NMI UC and the prospect of a 3-monthly intensive surveillance scheme. Our findings, based on a population of patients just diagnosed with primary or recurrent UC, are compared to age and gender matched healthy population surveys. Our results indicate that although 61% (87/142) of the patients reported to be sexually active, more than half (54%) of these patients perceive some degree of erectile dysfunction or vaginal lubrication problems. Percentages that evidently differ from calculations in age and gender matched healthy populations. Furthermore we found that almost one fourth (23%) of the sexually active participants was afraid to inflict harm to their partner in sexual contact. Risk factors such as gender, age, duration of bladder cancer diagnosis, number of recurrences, treatment, and marital status could not predict sexual activity or dysfunction.

Lindau et al report the prevalence of sexual activity in the United States to decline with age[15]. In a national probability sample of 3005 adults (1550 women, 1455 men) she reported 73% activity among respondents in the age of 57 to 64 years, 53% in the age of 65-74 years, and 26% who were 75-85 years of age, disregarding gender or the presence of partnership. Beutel et al showed, in a representative sample of a German population of 1271 women and 1070 men, that sexual activity is strongly dependent on age, gender and the presence of a partner[16]. The latter reported the prevalence of sexual activity according to gender in the presence of a partner; 93% and 84% younger than 60 years of age, 79% and 63% between the age of 61-70 years and 54% and 31% if older than 70 years of age, in males and females respectively. If we respect the presence of a partner and categorize age in our series of 142 adults (37 women, 105 men), we found a prevalence of sexual activity of 72% and 72%, of 88% and 63% and 53% and 29%, in males and females respectively. In comparison to the adults younger than 60 years of age in the healthy German population, the young patients with NMI UC in our study clearly showed less sexual activity. A possible explanation could be a poor general physical condition of the bladder cancer patients (i.e. 78% (29/37) of these

young patients are smokers). Men and women who rated their health as being poor were less likely to be sexually active and, respondents who answered to be sexually active, were more likely to report sexual problems[15]. This is confirmed in our series, we found the lowest health state in patients aged 51-60 years (p=.02 by One way ANOVA test). Patients who are sexually active perceive a significantly higher state of general health.

Sexual dysfunction has been evaluated in various studies. Laumann et al reported a higher prevalence in females (43%) than in males (31%), besides he showed that the experience of sexual dysfunction is more likely in patients with poor physical and emotional health[17]. Nicolosi et al collected data from 27,500 men and women in 29 countries and showed 10% erectile difficulties in men and 16% lubrication difficulties among women[18]. Lewis et al reported that 20-30% of adult men and 40-45% of adult women have at least one manifestation of sexual dysfunction[19]. Furthermore he described that concurrence of a genitourinary disease, next to other risk factors like presence of diabetes and smoking behaviour, are associated with sexual dysfunction. A large-scale multinational survey in the US and six European countries showed that LUTS (lower urinary tract symptoms) is an independent risk factor for sexual dysfunction in males, 49% of the males reported some degree of erectile dysfunction[20]. In the first 3 months after diagnosis, we found in our series of bladder cancer patients under surveillance a much higher rate of erectile and vaginal dysfunction rate as ever been described in previous studies, 58% and 78% in males and females respectively.

This study is the first to report that patients with bladder cancer are afraid to inflict harm to their partner by sexual contact. The patients in our series were evaluated for their sexual performance within three months after diagnosis of UC. Experiencing this fear was not related to a history of previous recurrences, however we found a positive effect of treatment by intra-vesical instillations on the degree of fear to harm the partner. Sexually active patients with a status of a long-term surveillance (multiple recurrences) treated with intravesical instillations tended to show less fear to harm their partner compared to long-term monitored patients without intra-vesical treatment (respectively 0/7 versus 5/14 patients with multiple recurrences experienced fear, p=.09), implying that treatment could have a positive effect on the sexual function of patients under surveillance with NMI UC. Many studies report about the fear for sexual contact as a known cause for avoidance of intercourse in patients with chronic or oncological diseases. These patients and possibly their partners fear an increased risk for cardiac arrest in cardiovascular diseases or acquiring infections in onco-hematological diseases[21, 22]. It is presumable that bladder cancer patients and their partners consider themselves protected in sexual contact after treatment with intra-vesical instillations. However, we cannot conclude this from the evaluation of our series.

As one of the very few studies in the literature, Stav et al evaluated the use of rigid cystoscopic examination in a longitudinal study and analysed sexual performance[14]. Fifty-one percent of the patients were sexually active (45 men and 6 women). The study was performed on a heterogeneous patient group, 62% (69/112) of the patients was under surveillance for bladder cancer. The authors reported a transient impair of libido (56% and 50% in males and females respectively) and satisfaction from sexual intercourse (77% of the sexually active patients) in patients monitored by rigid cystoscopy (in males and females) in the first two weeks after examination compared to pre-cystoscopic evaluation. Libido and satisfaction from sexual intercourse returned to baseline after one month. None of the parameters was influenced by gender or findings at examination. Unfortunately the authors did not report the prevalence of sexual dysfunction. In our NMI UC series only females were monitored by rigid cystoscopy, male patients were examined by flexible 17Ch cystoscope. It is conceivable that the findings regarding rigid cystoscopy can be compared to flexible cystoscopic examination.

Each bladder cancer patient in this series was questioned under identical circumstances in the course of surveillance. The cross-sectional analysis showed the prevalence of sexual function at that time-specific moment after bladder cancer diagnosis and before the start of an intensive surveillance scheme. However, several questions remain unanswered. Whether the sexual function is impaired by the diagnosis or by the impact of surveillance must be answered in future longitudinal surveys. Besides, we report the unique finding that a considerable amount of patients is mistakenly afraid to harm their partner by sexual contact; the present report however does not elaborate on the fear to inflict harm to a partner caused by being a bladder cancer patient and with the prospect of intensive surveillance by cystoscopy. This latter question will be answered by extended questionnaires.

We found a larger proportion of sexual dysfunction in our NMI bladder cancer patient group (54%) as ever been described. No risk factors were recognized. This problem warrants recognition as a significant public health concern. Diagnosis of NMI UC can be considered as a diagnosis of a chronic disease cause of the necessity to intensive long-term monitoring. Therefore the long-term sexual performance of these patients and partners would be likely to benefit from proper sexual information in the outpatient clinic.

References

1. Sengupta N, Siddiqui E, Mumtaz FH. Cancers of the bladder. J R Soc Health 2004:**124**:228-9.
2. Allard P, Bernard P, Fradet Y, Tetu B. The early clinical course of primary Ta and T1 bladder cancer: a proposed prognostic index. Br J Urol 1998: **81**:692-8.
3. Witjes JA, Melissen DO, Kiemeney LA. Current practice in the management of superficial bladder cancer in the Netherlands and Belgian Flanders: a survey. Eur Urol 2006:**49**:478-84.
4. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. Pharmacoeconomics 2003: **21**:1315-30.
5. Wright MP, Jones DJ. Surveillance for bladder cancer: the management of 4.8 million people. South-west Urologists. BJU Int 2000: **85**: 431-3.
6. Mariappan P, Smith G. A surveillance schedule for G1Ta bladder cancer allowing efficient use of check cystoscopy and safe discharge at 5 years based on a 25-year prospective database. J Urol 2005: **173**:1108-11.
7. Van Der Aa MNM, Steyerberg EW, Sen EF, Zwarthoff EC, Kirkels WJ, van der Kwast Th, et al. Patients' perceived burden of cystoscopic and urinary surveillance of bladder cancer: a randomized comparison. BJU Int 2008: **101**:1106-10.
8. Vriesema JL, Poucki MH, Kiemeney LA, Witjes JA. Patient opinion of urinary tests versus flexible urethrocystoscopy in follow-up examination for superficial bladder cancer: a utility analysis. Urology 2000: **56**:793-7.
9. Muezzinoglu T, Ceylan Y, Temeltas G, Lekili M, Buyuksu C. Evaluation of pain caused by urethrocystoscopy in patients with superficial bladder cancer: a perspective of quality of life. Onkologie 2005: **28**:260-4.
10. Kortmann BB, Sonke GS, D'ancona FC, Floratos DL, Debruyne FM, De La Rosette JJ. The tolerability of urodynamic studies and flexible cysto-urethroscopy used in the assessment of men with lower urinary tract symptoms. BJU Int 1999:**84**:449-53.
11. Denholm SW, Conn IG, Newsam JE, Chisholm GD. Morbidity following cystoscopy: comparison of flexible and rigid techniques. Br J Urol 1990:**66**:152-4.
12. Herr HW, Schneider M. Outpatient flexible cystoscopy in men: a randomized study of patient tolerance. J Urol 2001:**165**:1971-2.
13. Allareddy V, Kennedy J, West MM, Konety BR. Quality of life in long-term survivors of bladder cancer. Cancer 2006:**106**:2355-62.
14. Stav K, Leibovici D, Goren E, Livshitz A, Siegel YI, Lindner A, et al. Adverse effects of cystoscopy and its impact on patients' quality of life and sexual performance. Isr Med Assoc J 2004: **6**:474-8.
15. Lindau ST, Schumm LP, Laumann EO, Levinson W, O'muircheartaigh CA, Waite LJ. A study of sexuality and health among older adults in the United States. N Engl J Med 2007: **357**:762-74.
16. Beutel ME, Weidner W, Brahler E. Epidemiology of sexual dysfunction in the male population. Andrologia 2006: **38**:115-21.
17. Laumann EO, Paik A, Rosen RC. Sexual dysfunction in the United States: prevalence and predictors. Jama 1999: **281**:537-44.
18. Nicolosi A, Laumann EO, Glasser DB, Moreira ED, Jr., Paik A, Gingell C. Sexual behavior and sexual dysfunctions after age 40: the global study of sexual attitudes and behaviors. Urology 2004: **64**:991-7.
19. Lewis RW, Fugl-Meyer KS, Bosch R, Fugl-Meyer AR, Laumann EO, Lizza E, et al. Epidemiology/risk factors of sexual dysfunction. J Sex Med 2004: **1**:35-9.
20. Rosen R, Altwein J, Boyle P, Kirby RS, Lukacs B, Meuleman E, et al. Lower urinary tract symptoms and male sexual dysfunction: the multinational survey of the aging male (MSAM-7). Eur Urol 2003: **44**:637-49.
21. Mandras SA, Uber PA, Mehra MR. Sexual activity and chronic heart failure. Mayo Clin Proc 2007: **82**:1203-10.
22. Melo Ade S, Carvalho EC, Pela NT. The sexuality of patients with onco-hematological diseases. Rev Lat Am Enfermagem 2006: **14**:227-32.

APPENDIX

Questionnaires I-IV

Questionnaire I

Patients received simultaneously 8 copies of the following questionnaire together with the announcement in which trial arm they were randomized, a patient information guide and another questionnaire. Patients randomized in the Cystoscopy-arm were asked to fill out the following questionnaire concerning pain and discomfort in the first week after they underwent cystoscopic examination, they were asked to repeat the questionnaire every time they underwend cystoscopic examination.

Pain and discomfort questionnaire addressed to patients in the cystoscopy-arm

Een week geleden bent u op de polikliniek urologie geweest voor onderzoek van de blaas (cystoscopie). Met een cystoscoop is toen in uw blaas gekeken. Als gevolg van een cystoscopie kunnen bij sommige mensen bepaalde klachten optreden. Daarover gaan de volgende vragen.

Vragen 1,2 en 3 gaan over het bezoek aan de polikliniek 1 week geleden; direct voor, tijdens en direct na cystoscopie. Kruis op elke regel het antwoord aan dat het best op u van toepassing is:

1.	**Hoe vervelend vond U:**	**niet vervelend**	**vrij vervelend**	**zeer vervelend**
a.	de voorbereiding voor de cystoscopie	☐	☐	☐
b.	het inbrengen van de cystoscoop	☐	☐	☐
c.	het ondergaan van de cystoscopie	☐	☐	☐
d.	de uren direct ná de cystoscopie	☐	☐	☐
2.	**Hoe pijnlijk vond U:**	**niet pijnlijk**	**vrij pijnlijk**	**zeer pijnlijk**
a.	de voorbereiding voor de cystoscopie	☐	☐	☐
b.	het inbrengen van de cystoscoop	☐	☐	☐
c.	het ondergaan van de cystoscopie	☐	☐	☐
d.	de uren direct ná de cystoscopie	☐	☐	☐
3.	**Hoe tevreden was / bent u met:**	**niet tevreden**	**vrij tevreden**	**zeer tevreden**
a.	de ontvangst op de polikliniek	☐	☐	☐
b.	de wachttijd op de polikliniek	☐	☐	☐
c.	de uitleg op de polikliniek	☐	☐	☐

Vragen 4 tot en met 12 gaan over de periode 1 week na cystoscopie. Beantwoord elke vraag door één hokje aan te kruisen. Als u niet zeker weet welk antwoord u moet geven, geef dan het best mogelijke antwoord.

4. Heeft u in de week na de cystoscopie pijn gehad bij het plassen?

☐ Nee

☐ Ja, maar dat was binnen een week over

☐ Ja, en dat heb ik nog steeds

5. Heeft u in de week na cystoscopie het gevoel gehad vaak maar kleine beetjes te kunnen plassen?

☐ Nee

☐ Ja, maar dat was binnen een week over

☐ Ja, en dat heb ik nog steeds

6. Heeft u in de afgelopen week pijnstillers gebruikt in verband met pijn als gevolg van de cystoscopie ?

☐ Nee, niet nodig

☐ Ja **en** dat heeft geholpen

☐ Ja, **maar** het helpt niet

7. Heeft u in de week na de cystoscopie antibiotica geslikt?

☐ Nee

☐ Ja

8. Heeft u in de week na de cystoscopie koorts (boven 38°C) gehad?

☐ Nee

☐ Ja, gedurende dagen *(in het vierkantje hebt u ruimte om het aantal dagen in te vullen)*

9. Heeft u in de week na de cystoscopie bloed in de urine gehad?

☐ Nee

☐ Ja, maar de urine kleurde lichtrood

☐ Ja, er zat veel bloed in de urine

10. Heeft u in verband met de cystoscopie in de afgelopen week de huisarts bezocht?

☐ Nee

☐ Ja

11. Hebben klachten als gevolg van de cystoscopie u gedurende de AFGELOPEN WEEK gehinderd bij uw **dagelijkse bezigheden** (zoals werk buitenshuis, huishoudelijk werk)?

Helemaal niet	**Een klein beetje**	**Nogal**	**Veel**	**Heel erg veel**
☐	☐	☐	☐	☐

12. Hebben klachten als gevolg van de cystoscopie u gedurende de AFGELOPEN WEEK gehinderd bij uw **sociale leven** (zoals vrienden of familie bezoeken, etc.)?

Helemaal niet	**Een klein beetje**	**Nogal**	**Veel**	**Heel erg veel**
☐	☐	☐	☐	☐

Questionnaire II

Patients received simultaneously 5 copies of the following questionnaire together with the announcement in which trial arm they were randomized, a patient information guide and another questionnaire. Patients randomized in the MA-arm were asked to fill out the following questionnaire concerning pain and discomfort in the first week after they went to the outpatient clinic for the result of the urinary test, they were asked to repeat this questionnaire every time a urinary test was performed. Note: patients randomized in the MA-arm received at the same time 5 questionnaires concerning pain and discomfort after cystoscopic examination.

Pain and discomfort questionnaire addressed to patients in the MA-arm

Ongeveer drie weken geleden bent u op de polikliniek urologie geweest, en heeft u urine ingeleverd voor onderzoek van de blaas. Ongeveer een week geleden bent u op de polikliniek geweest voor de uitslag van de urinetest of heeft u telefonisch contact gehad met uw uroloog over de uitslag van de urinetest. Hierover gaan de volgende vragen.

Vragen 1 en 2 gaan over het bezoek aan de polikliniek 1 week geleden; kruis op elke regel het antwoord aan dat het best op u van toepassing is:

1.	**Hoe vervelend vond U:**	**niet vervelend**	**vrij vervelend**	**zeer vervelend**
e.	het opvangen van urine	☐	☐	☐
f.	het brengen van urine naar de poli	☐	☐	☐
g.	het wachten op de uitslag	☐	☐	☐

2.	**Hoe tevreden was u met::**	**niet tevreden**	**vrij tevreden**	**zeer tevreden**
d.	de ontvangst op de polikliniek	☐	☐	☐
e.	de wachttijd op de polikliniek	☐	☐	☐
f.	de uitleg op de polikliniek	☐	☐	☐

Vragen 3 tot en met 11 gaan over de afgelopen week. Beantwoord elke vraag door één hokje aan te kruisen. Als u niet zeker weet welk antwoord u moet geven, geef dan het best mogelijke antwoord.

3. Heeft u de afgelopen week pijn gehad bij het plassen?

☐ Nee

☐ Ja, maar dat was binnen een week over

☐ Ja, en dat heb ik nog steeds

4. Heeft u de afgelopen week het gevoel gehad vaak maar kleine beetjes te kunnen plassen?

☐ Nee

☐ Ja, maar dat was binnen een week over

☐ Ja, en dat heb ik nog steeds

5. Heeft u de afgelopen week pijnstillers gebruikt in verband met uw blaasaandoening?

☐ Nee, niet nodig

☐ Ja en dat heeft geholpen

☐ Ja, maar het helpt niet

6. Heeft u de afgelopen week antibiotica geslikt?

☐ Nee

☐ Ja

7. Heeft u de afgelopen week koorts (boven 38°C) gehad?

☐ Nee

☐ Ja, gedurende dagen (in het vierkantje hebt u ruimte om het aantal dagen in te vullen)

8. Heeft u de afgelopen week bloed in de urine gehad?

☐ Nee

☐ Ja, maar de urine kleurde lichtrood

☐ *Ja, er zat veel bloed in de urine*

9. Heeft u de afgelopen week de huisarts bezocht in verband met blaasklachten?

☐ Nee

☐ Ja

10. Heeft u gedurende de AFGELOPEN WEEK klachten gehad die in verband staan met uw blaasaandoening en u gehinderd hebben bij uw dagelijkse bezigheden (zoals werk buitenshuis, huishoudelijk werk)?

Helemaal niet	Een klein beetje	Nogal	Veel	Heel erg veel
☐	☐	☐	☐	☐

11. Heeft u gedurende de AFGELOPEN WEEK klachten gehad die in verband staan met uw blaasaandoening en u gehinderd hebben bij uw sociale leven (zoals vrienden of familie bezoeken, etc.)?

Helemaal niet	Een klein beetje	Nogal	Veel	Heel erg veel
☐	☐	☐	☐	☐

Questionnaire III

Patients received the following questionnaire together with 8 questionnaires concerning pain and discomfort, a patient information guide and the announcement in which trial arm they were randomized. They were asked to instantly fill out the questionnaire and send it back as soon as possible.

Zet bij iedere vraag in de lijst hieronder een kruisje in het hokje voor de zin die past bij uw gezondheidstoestand van VANDAAG.

1. Mobiliteit

- ☐ Ik heb geen problemen met lopen
- ☐ Ik heb enige problemen met lopen
- ☐ Ik ben bedlegerig

2. Zelfzorg

- ☐ Ik heb geen problemen om mijzelf te wassen of aan te kleden
- ☐ Ik heb enige problemen om mijzelf te wassen of aan te kleden
- ☐ Ik ben niet in staat mijzelf te wassen of aan te kleden

3. Dagelijkse activiteiten
(bijv. werk, studie, huishouden, gezins- en vrijetijdsactiviteiten)

- ☐ Ik heb geen problemen met mijn dagelijkse activiteiten
- ☐ Ik heb enige problemen met mijn dagelijkse activiteiten
- ☐ Ik ben niet in staat mijn dagelijkse activiteiten uit te voeren

4. Pijn / klachten

- ☐ Ik heb geen pijn of andere klachten
- ☐ Ik heb matige pijn of andere klachten
- ☐ Ik heb zeer ernstige pijn of andere klachten

5. Stemming

- ☐ Ik ben niet angstig of somber
- ☐ Ik ben matig angstig of somber
- ☐ Ik ben erg angstig of somber

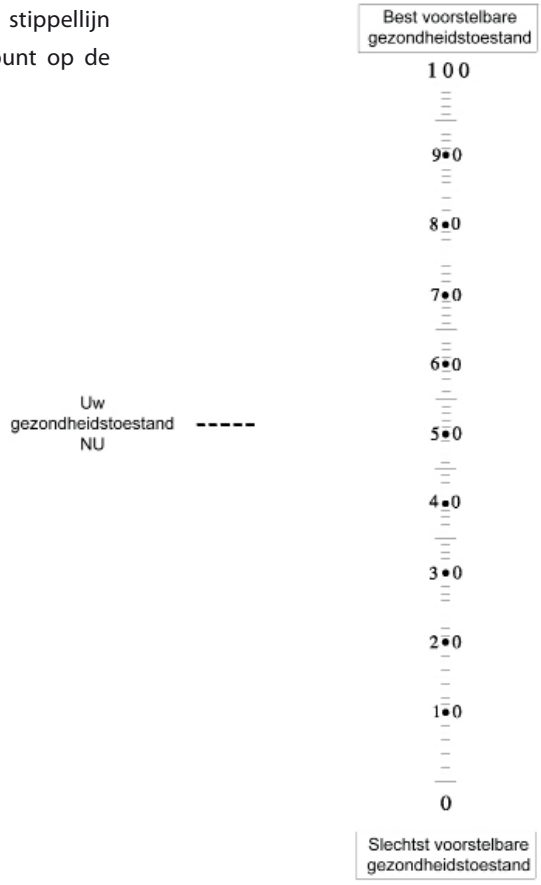
Gezondheidsmeetschaal

Wij willen u vragen op een meetschaal (thermometer) aan te geven hoe goed of hoe slecht volgens u uw gezondheidstoestand NU is.

Op de thermometer hieronder betekent '100' de beste gezondheidstoestand die men zich kan voorstellen, en '0' de slechtste gezondheidstoestand die men zich kan voorstellen.

6. **Teken nu een pijl** vanaf "UW GEZONDHEIDSTOESTAND NU" naar het punt op de thermometer dat volgens u aangeeft hoe goed of hoe slecht uw gezondheidstoestand NU is.

Laat de pijl beginnen bij de stippellijn en trek een lijn naar een punt op de Thermometer.



Wij zijn geïnteresseerd in uw lichamelijke en emotionele toestand gedurende de AFGELOPEN WEEK. Wilt u alle vragen beantwoorden door op elke regel het getal te omcirkelen dat het meest op u van toepassing is.

Gedurende de afgelopen week: (omcirkel 1 cijfer op elke regel)

		Helemaal Niet	Een beetje	Nogal	Heel erg
7.	Heeft u moeite gehad met het doen van inspannende activiteiten zoals bijv. het dragen van een zware boodschappentas en/of een koffer?	1	2	3	4
8.	Heeft u moeite gehad met het maken van een **lange** wandeling?	1	2	3	4
9.	Heeft u moeite gehad met het maken van een **korte** wandeling, buitenshuis?	1	2	3	4
10.	Was het nodig overdag in bed / een stoel te blijven?	1	2	3	4
11.	Heeft u hulp nodig gehad met eten, aankleden, uzelf te wassen of naar het toilet gaan?	1	2	3	4
12.	Was u beperkt bij het doen van uw werk of andere dagelijkse bezigheden?	1	2	3	4
13.	Was u beperkt in het uitoefenen van uw hobby's of bij andere bezigheden die u in uw vrije tijd doet?	1	2	3	4
14.	Was u kortademig?	1	2	3	4
15.	Heeft u pijn gehad?	1	2	3	4
16.	Heeft u behoefte gehad te rusten?	1	2	3	4
17.	Heeft u moeite met slapen gehad?	1	2	3	4
18.	Heeft u zich slap gevoeld?	1	2	3	4
19.	Heeft u gebrek aan eetlust gehad?	1	2	3	4
20.	Heeft u zich misselijk gevoeld?	1	2	3	4
21.	Heeft u overgegeven?	1	2	3	4
22.	Heeft u last gehad van obstipatie? (Was u verstopt?)	1	2	3	4
23.	Heeft u last gehad van diarree?	1	2	3	4

Gedurende de afgelopen week: (omcirkel 1 cijfer op elke regel)

		Helemaal Niet	Een beetje	Nogal	Heel erg
24.	Bent u moe geweest?	1	2	3	4
25.	Heeft pijn u gehinderd in uw dagelijkse bezigheden?	1	2	3	4
26.	Heeft u moeite gehad met het concentreren op dingen, zoals een krant lezen of televisie kijken?	1	2	3	4
27.	Voelde u zich gespannen?	1	2	3	4
28.	Heeft u zich zorgen gemaakt?	1	2	3	4
29.	Heeft u zich prikkelbaar gevoeld?	1	2	3	4
30.	Heeft u zich neerslachtig gevoeld?	1	2	3	4
31.	Heeft u moeite gehad met het herinneren van dingen?	1	2	3	4
32.	Heeft uw lichamelijke toestand of medische behandeling uw familieleven in de weg gestaan?	1	2	3	4
33.	Heeft uw lichamelijke toestand of medische behandeling u belemmerd in uw sociale bezigheden?	1	2	3	4
34.	Heeft uw lichamelijke toestand of medische behandeling financiële moeilijkheden met zich meegebracht?	1	2	3	4

Wilt u voor de volgende vragen een getal tussen 1 en 7 omcirkelen dat het meest op u van toepassing is.

35. Hoe zou u uw algehele gezondheid op dit moment beoordelen?

1	2	3	4	5	6	7
Erg slecht						Uitstekend

36. Hoe zou u uw algehele 'kwaliteit van leven' op dit moment beoordelen?

1	2	3	4	5	6	7
Erg slecht						Uitstekend

Patiënten melden ons soms dat zij de volgende klachten of problemen hebben. Gelieve aan te duiden in welke mate u deze symptomen of problemen heeft ondervonden gedurende de afgelopen week. Gelieve op elke regel het cijfer te omcirkelen dat op u van toepassing is.

Gedurende de afgelopen week: (omcirkel 1 cijfer op elke regel)

		Helemaal Niet	Een beetje	Nogal	Heel erg
37.	Moest u **overdag** vaak plassen?	1	2	3	4
38.	Moest u **'s nachts** vaak plassen?	1	2	3	4
39.	Toen u voelde dat u moest plassen, moest u zich dan haasten om naar het toilet te gaan?	1	2	3	4
40.	Kon u niet voldoende slapen, omdat u 's nachts vaak naar het toilet moest?	1	2	3	4
41.	Kon u moeilijk het huis verlaten omdat u altijd dicht in de buurt van een toilet moest blijven?	1	2	3	4
42.	Had u onvrijwillig urineverlies (lekken van urine?)	1	2	3	4
43.	Had u pijn of een brandend gevoel toen u plaste?	1	2	3	4
44.	Had u koorts?	1	2	3	4
45.	Voelde u zich ziek of onwel?	1	2	3	4
46.	Was het moeilijk uw leven te organiseren rond de herhaalde afspraken voor behandelingen van de blaas (cystoscopieën of instillaties)?	1	2	3	4
47.	Maakte u zich zorgen over het feit dat u meermaals behandelingen van de blaas moest ondergaan (cystoscopieën of instillaties)?	1	2	3	4
48.	Maakte u zich zorgen over uw gezondheid in de toekomst?	1	2	3	4
49.	Maakte u zich zorgen over de resultaten van de tests en onderzoeken?	1	2	3	4
50.	Maakte u zich zorgen over eventuele behandelingen in de toekomst?	1	2	3	4
51.	Had u een opgeblazen gevoel in de buik?	1	2	3	4
52.	Had u last van winderigheid?	1	2	3	4

Gedurende de afgelopen 4 weken: (omcirkel 1 cijfer op elke regel)

		Helemaal Niet	Een beetje	Nogal	Heel erg
53.	Hoeveel **zin in seks** had u?	1	2	3	4
54.	In welke mate was u seksueel actief (met of zonder geslachtsgemeenschap)?	1	2	3	4
55.	**Enkel voor mannen:** Kon u moeilijk een erectie krijgen of behouden?	1	2	3	4
56.	**Enkel voor mannen:** Had u problemen met de zaadlozing (bijv. klaarkomen zonder zaadlozing)?	1	2	3	4

De onderstaande 4 vragen alleen beantwoorden indien u seksueel actief was gedurende de afgelopen 4 weken:

(omcirkel 1 cijfer op elke regel)

		Helemaal Niet	Een beetje	Nogal	Heel erg
57.	Maakte de gedachte aan seksueel intiem worden, u ongemakkelijk?	1	2	3	4
58.	Was u bang dat u, tijdens het seksueel contact, uw partner zou kunnen schaden door de behandelingen die u voor de blaas onderging?	1	2	3	4
59.	In welke mate heeft u van seks genoten?	1	2	3	4
60.	**Enkel voor vrouwen:** had u een droge vagina of andere problemen tijdens de geslachtsgemeenschap?	1	2	3	4

Hieronder vindt u vragen over de kans dat er bij u weer een blaaspoliep zal worden gevonden. We willen graag van u weten hoe u daarover denkt. Kruis de mogelijkheden aan die voor u het meest van toepassing is.

61. Hoe groot schat u de kans dat er binnen 3 jaar weer een blaaspoliep bij u zal worden gevonden? De kans dat er weer een blaaspoliep bij mij wordt gevonden in de komende 3 jaar is:

- ☐ Minder dan 5%
- ☐ Tussen 5 en 20%
- ☐ Tussen 20 en 40%
- ☐ Tussen 40 en 60%
- ☐ Tussen 60 en 80%
- ☐ Meer dan 80% maar minder dan 100%
- ☐ Absoluut zeker, namelijk 100%

62. Hoe zou u deze kans omschrijven als u op uw gevoel afgaat ? Ik ervaar de kans dat er bij mij binnen 3 jaar weer een blaaspoliep wordt gevonden als:

- ☐ erg klein
- ☐ klein
- ☐ redelijk klein
- ☐ niet klein en niet groot
- ☐ redelijk groot
- ☐ groot
- ☐ erg groot

63. Hoe erg zou u het vinden als er bij u weer een blaaspoliep zou worden gevonden? (geef d.m.v. van een kruisje op de balk aan, hoe erg u dit zult vinden)

0% 100%

64. Hoe groot schat u de kans dat er bij een eerstvolgende controle weer een blaaspoliep bij u zal worden gevonden? De kans dat er bij de eerstvolgende controle een blaaspoliep wordt gevonden schat ik op:

- ☐ Heel waarschijnlijk minder dan 5%
- ☐ Ongeveer 5%
- ☐ Ongeveer 10%
- ☐ Ongeveer 25%
- ☐ Tussen 30 en 40%
- ☐ Tussen 40 en 50%
- ☐ Tussen 50 en 75%
- ☐ Tussen 75 en 99%
- ☐ Absoluut zeker, namelijk 100%

Gaat u er voor de volgende vraag vanuit dat cystoscopie en urineonderzoek precies even betrouwbaar zijn voor het opsporen van blaaspoliepen.

65. Als de betrouwbaarheid van cystoscopie en urineonderzoek precies gelijk is en u mag kiezen, voor welk onderzoek zou u dan kiezen?

- ☐ zeker de cystoscopie
- ☐ waarschijnlijk de cystoscopie
- ☐ geen voorkeur
- ☐ waarschijnlijk het urineonderzoek
- ☐ zeker het urineonderzoek

De volgende vragen dienen er voor om te weten te komen hoe u zich emotioneel voelt. Lees iedere vraag en geef uw antwoord aan met een kruisje in het hokje dat het best weergeeft hoe u zich gedurende de afgelopen week gevoeld heeft. Denk niet te lang na

over uw antwoord. Het gaat bij al deze vragen over uw eigen indruk. Er bestaan geen foute antwoorden, elk antwoord is goed, als het maar uw eigen indruk weergeeft.

66. Ik voel me gespannen

- ☐ Meestal
- ☐ Vaak
- ☐ Af en toe, soms
- ☐ Helemaal niet

67. Ik geniet nog steeds van de dingen waar ik vroeger van genoot

- ☐ Zeker zo veel
- ☐ Niet zoveel als vroeger
- ☐ Weinig
- ☐ Haast helemaal niet

68. Ik krijg een soort angstgevoel alsof er elk moment iets vreselijks kan gebeuren

- ☐ Heel zeker en vrij erg
- ☐ Ja, maar niet zo erg
- ☐ Een beetje, maar ik maak me er geen zorgen over
- ☐ Helemaal niet

69. Ik kan lachen en de dingen van de vrolijke kant zien

- ☐ Net zoveel als vroeger
- ☐ Niet zo goed als vroeger
- ☐ Beslist niet zoveel als vroeger
- ☐ Helemaal niet

70. Ik maak me vaak ongerust

- ☐ Heel erg vaak
- ☐ Vaak
- ☐ Af en toe maar niet vaak
- ☐ Alleen soms

71. Ik voel me opgewekt

- ☐ Helemaal niet
- ☐ Niet vaak
- ☐ Soms
- ☐ Meestal

72. Ik kan rustig zitten en me ontspannen

- ☐ Zeker
- ☐ Meestal
- ☐ Niet vaak
- ☐ Helemaal niet

73. Ik voel alsof alles moeizamer gaat

- ☐ Bijna altijd
- ☐ Heel vaak
- ☐ Soms
- ☐ Helemaal niet

74. Ik krijg een soort benauwd, gespannen gevoel in mijn maag

- ☐ Helemaal niet
- ☐ Soms
- ☐ Vrij vaak
- ☐ Heel vaak

75. Ik heb geen interesse meer in mijn uiterlijk

- ☐ Zeker
- ☐ Niet meer zoveel als zou moeten
- ☐ Waarschijnlijk niet zoveel
- ☐ Evenveel interesse als vroeger

76. Ik heb helemaal geen rust en voel dat ik iets te doen moet hebben

- ☐ Heel erg
- ☐ Tamelijk veel
- ☐ Niet erg veel
- ☐ Helemaal niet

77. Ik verheug me van tevoren al op dingen

- ☐ Net zoveel als vroeger
- ☐ Een beetje minder dan vroeger
- ☐ Zeker minder dan vroeger
- ☐ Bijna nooit

78. Ik krijg plotseling gevoelens van panische angst

- ☐ Zeer vaak
- ☐ Tamelijk vaak
- ☐ Niet erg vaak
- ☐ Helemaal niet

79. Ik kan van een goed boek genieten, of van een radio- of televisieprogramma

- ☐ Vaak
- ☐ Soms
- ☐ Niet zelden
- ☐ Heel zelden

Probeert u zich voor te stellen dat een cystoscopie wel in uw eigen ziekenhuis kan plaatsvinden maar het urineonderzoek niet. Als u liever het urineonderzoek wilt laten doen moet u de urine voor het urineonderzoek ZELF naar een ander ziekenhuis brengen. Het wegbrengen van de urine kost u reistijd (de kosten maken niet uit; de verzekering zou de extra reiskosten vergoeden).

80. Hoeveel uren reistijd bent u bereid in totaal (heen en terugreis) te reizen voor een urineonderzoek in plaats van een cystoscopie?

- ☐ 0 uur
- ☐ 1 uur
- ☐ 2 uur
- ☐ 3 uur
- ☐ 4 uur
- ☐ 5-6 uur
- ☐ 7-8 uur
- ☐ 9-10 uur
- ☐ meer dan 10 uur

Stelt u zich nu voor dat u na een cystoscopie direct hoort of u wel of geen nieuwe poliepen heeft, maar dat u op de uitslag van urineonderzoek een aantal dagen moet wachten. Als u dus direct de uitslag wilt hebben, zou u moeten kiezen voor cystoscopie.

81. Hoeveel dagen zou u bereid zijn te wachten op de uitslag van een urineonderzoek?

- ☐ 0 dagen
- ☐ 1 dag
- ☐ 2-3 dagen
- ☐ 4-7 dagen
- ☐ 8-10 dagen
- ☐ 11-14 dagen
- ☐ meer dan 14 dagen

82. Tenslotte; het urineonderzoek kan om technische redenen mislukken. Om met zekerheid te kunnen vaststellen of uw blaaskanker is teruggekomen, zal worden geadviseerd alsnog een cystoscopie te ondergaan (1 op de 20 urinetesten kan mislukken) Zou u het dan erg vinden om alsnog een cystoscopie te ondergaan :

Ja, omdat ……
……
……

Nee, omdat ……
……
……

Hieronder vindt u een aantal uitspraken die mensen kunnen doen over gedachten en gevoelens omtrent blaaskanker. Bekijk de uitspraken één voor één. U kunt in de hokjes aankruisen in hoeverre elke uitspraak op u van toepassing was tijdens de afgelopen 7 dagen. Als de uitspraak niet van toepassing is, zet u een kruisje bij 'helemaal niet'.

83.		**Helemaal Niet**	**Zelden**	**Soms**	**Vaak**
a.	Ik moest aan blaaskanker denken zonder dat ik dat wilde.	☐	☐	☐	☐
b.	Iedere keer als ik aan blaaskanker herinnerd werd, deed ik mijn uiterste best om te voorkomen dat ik van streek zou raken	☐	☐	☐	☐
c.	Ik deed mijn best om gedachten aan blaaskanker uit te bannen	☐	☐	☐	☐
d.	Omdat ik steeds aan blaaskanker moest denken sliep ik slecht.	☐	☐	☐	☐
e.	Ik kon overspoeld raken door mijn gedachten aan blaaskanker.	☐	☐	☐	☐
f.	Ik heb over blaaskanker gedroomd.	☐	☐	☐	☐
g.	Ik ging mensen of situaties uit de weg die me aan blaaskanker deden denken.	☐	☐	☐	☐
h.	Ik heb er zo'n onwerkelijk gevoel over, alsof er niks aan de hand is.	☐	☐	☐	☐
i.	Ik heb mijn uiterste best gedaan niet over blaaskanker te praten.	☐	☐	☐	☐
j.	Beelden over blaaskanker konden zomaar in mijn gedachten schieten.	☐	☐	☐	☐
k.	Er gebeurde aldoor wel iets waardoor ik er plotseling weer aan herinnerd werd.	☐	☐	☐	☐
l.	Ik wist wel dat ik nog steeds veel gevoelens erover had, maar ik wil er liever niet aan denken.	☐	☐	☐	☐
m.	Ik wilde gewoon niet aan blaaskanker denken.	☐	☐	☐	☐
n.	Als ik aan blaaskanker moest denken, kwamen meteen weer alle gevoelens erover terug.	☐	☐	☐	☐
o.	Mijn gevoelens over blaaskanker waren als het ware verdoofd.	☐	☐	☐	☐

84. OPLEIDING Wat is de hoogste opleiding die u met een diploma heeft afgesloten ?

- ☐ lager onderwijs (basisonderwijs)
- ☐ lager beroepsonderwijs (bijv. LTS, LHNO, huishoudschool, lager land- en tuinbouwonderwijs)
- ☐ middelbaar algemeen onderwijs (bijv. LAVO, ULO, MULO, MAVO, 3-jarige HBS)
- ☐ middelbaar beroepsonderwijs (bijv. MTS, MEAO)
- ☐ voortgezet algemeen onderwijs (bijv. HBS, MMS, gymnasium, HAVO, VWO)
- ☐ hoger beroepsonderwijs (bijv. HTS, HEAO, sociale academie, PABO, lerarenopleiding)
- ☐ wetenschappelijk onderwijs (universiteit)
- ☐ anders, nl……

85. BURGERLIJKE STAAT Wat is uw burgerlijke staat ?

- ☐ Gehuwd / geregistreerd partnerschap
- ☐ Ongehuwd en nooit gehuwd geweest
- ☐ Gescheiden
- ☐ Weduwe / weduwnaar

86. LEEFSITUATIE Welke situatie is op dit moment voor u van toepassing ?

- ☐ Ik woon samen met mijn echtgenoot / partner
- ☐ Ik woon samen met mijn echtgenoot / partner en kinderen
- ☐ Ik heb een duurzame relatie, maar we wonen niet samen
- ☐ Ik woon samen met kinderen
- ☐ Ik woon samen met mijn ouders
- ☐ Ik ben alleenstaand
- ☐ anders, nl……

87. WERK Welke situatie is voor u op dit moment het meest van toepassing ?

- ☐ Ik heb betaald werk
- ☐ Ik heb geen betaald werk
  - o want ik zorg voor de huishouding en evt. kinderen
  - o meer vanwege mijn blaasproblemen
  - o meer vanwege andere gezondheidsproblemen
  - o om andere redenen (bijv. onvrijwillig werkloos, vrijwilligerswerk, etc.)
- ☐ Ik ben gepensioneerd of met de VUT
- ☐ Ik ben scholier of student

88. Heeft u ooit gerookt ?

- ☐ Ja, zoveel jaar ……..
- ☐ Nee

89. Wat voor werk heeft u gedaan / doet u?

……..

Questionnaire IV

Patients were asked to fill out this questionnaire in the waiting room before cystoscopic examination.

Allereerst stellen wij u 3 algemene vragen

1. **Vult u eerst de datum in van vandaag:**

   _____

   (dag – maand – jaar; bijv. 01-04-05 voor 1 april 2005, binnen de lijntjes blijven a.u.b.)

2. **Uw geboortedatum:**

   _____

   (dag – maand – jaar; bijv. 01-04-05 voor 1 april 2005, binnen de lijntjes blijven a.u.b.)

3. **Uw geslacht:**

   Man / Vrouw (omcircel)

Het kan zijn dat u al meedoet aan een onderzoek naar "een nieuwe methode om uw blaas te controleren." U heeftdan vergelijkbare vragenlijsten thuis ontvangen, een aantal vragen zullen u bekend voorkomen. Wij willen u vragen om deze vragenlijst ook in vullen,

4. **Doet u al mee met het onderzoek "een nieuwe methode om uw blaas te controleren?" en ontvangt u thuis ook vragenlijsten**

   ☐ Ja, uw achternaam / initialen

   ☐ Nee

De volgende vragen gaan over de cystoscopie:

5. **Hoe vaak heeft u een cystoscopie gehad?**

   ……..

6. **Om welke reden dient u vandaag een cystoscopie te ondergaan?**

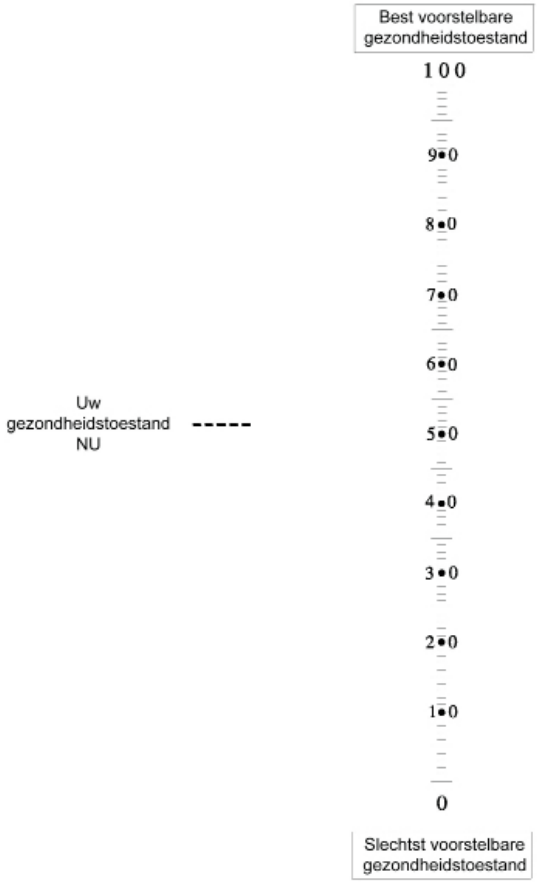
   ……
   ……..

Gezondheidsmeetschaal

We willen u vragen op een meetschaal (thermometer) aan te geven hoe goed of hoe slecht volgens u uw gezondheidstoestand NU is.

Op de thermometer hieronder betekent '100' de beste gezondheidstoestand die men zich kan voorstellen, en '0' de slechtste gezondheidstoestand die men zich kan voorstellen.

7. **Teken nu een pijl** vanaf "UW GEZONDHEIDSTOESTAND NU" naar het punt op de thermometer dat volgens u aangeeft hoe goed of hoe slecht uw gezondheidstoestand NU is. Laat de pijl beginnen bij de stippellijn.



Zelf-beoordelings vragenlijst

Hieronder vindt u een aantal uitspraken die mensen hebben gebruikt om zichzelf te beschrijven. Lees iedere uitspraak door en omcirkel op elke regel het cijfer rechts van die uitspraak om daarmee aan te geven hoe u zich nu voelt, dus nu op dit moment.
Er zijn geen goede of slechte antwoorden. Denk niet te lang na en geef uw eerste indruk, die is meestal de beste. Het gaat er dus om dat u weergeeft wat u op dit moment voelt.

Gedurende de afgelopen week: (omcirkel 1 cijfer op elke regel)

		Helemaal Niet	Een beetje	Nogal	Heel erg
8.	Ik voel me kalm	1	2	3	4
9.	Ik voel me veilig	1	2	3	4
10.	Ik ben gespannen	1	2	3	4
11.	Ik voel me onrustig	1	2	3	4
12.	Ik voel me op mijn gemak	1	2	3	4
13.	Ik ben in de war	1	2	3	4
14.	Ik pieker over nare dingen die kunnen gebeuren	1	2	3	4
15.	Ik voel me voldaan	1	2	3	4
16.	Ik ben bang	1	2	3	4
17.	Ik voel me aangenaam	1	2	3	4
18.	Ik voel me zeker	1	2	3	4
19.	Ik voel me nerveus	1	2	3	4
20.	Ik ben zenuwachtig	1	2	3	4
21.	Ik ben besluiteloos	1	2	3	4
22.	Ik ben ontspannen	1	2	3	4
23.	Ik voel me tevreden	1	2	3	4
24.	Ik maak me zorgen	1	2	3	4
25.	Ik voel me gejaagd	1	2	3	4
26.	Ik voel me evenwichtig	1	2	3	4
27.	Ik voel me prettig	1	2	3	4

Hoe vaak heeft u** in de afgelopen week, als gevolg van gedachten en gevoelens over de komende cystoscopie, **de volgende verschijnselen gehad?

Gedurende de afgelopen week: (omcirkel 1 cijfer op elke regel)

		Helemaal Niet	Een beetje	Nogal	Heel erg
28.	Had ik slaapproblemen	0	1	2	3
29.	Was mijn eetlust veranderd	0	1	2	3
30.	Was ik ongelukkig of neerslachtig	0	1	2	3
31.	Was ik bang en paniekerig	0	1	2	3
32.	Was ik nerveus en gestresst	0	1	2	3
33.	Voelde ik mij onder spanning staan	0	1	2	3
34.	Merkte ik dat ik dingen verzweeg voor degene die me na staan	0	1	2	3
35.	Merkte ik dat ik me afreageerde op anderen	0	1	2	3
36.	Merkte ik dat ik me duidelijk terugtrok van degenen die me na staan	0	1	2	3
37.	Had ik moeite met dingen die ik gewoonlijk in en om het huis doe	0	1	2	3
38.	Had ik moeite met het nakomen van mijn werk en andere verplichtingen	0	1	2	3
39.	Was ik bezorgd over de toekomst	0	1	2	3

De volgende vragen dienen er voor om te weten te komen hoe u zich emotioneel voelt. Lees iedere vraag en geef uw antwoord aan met een kruisje in het hokje dat het best weergeeft hoe u zich gedurende de afgelopen week tot op dit moment heeft gevoeld. Denk niet te lang na over uw antwoord. Het gaat bij al deze vragen over uw eigen indruk. Er bestaan geen foute antwoorden, elk antwoord is goed, als het maar uw eigen indruk weergeeft.

40. Ik voel me gespannen

- ☐ Meestal
- ☐ Vaak
- ☐ Af en toe, soms
- ☐ Helemaal niet

41. Ik geniet nog steeds van de dingen waar ik vroeger ook van genoot

- ☐ Zeker zo veel
- ☐ Niet zoveel als vroeger
- ☐ Weinig
- ☐ Haast helemaal niet

42. Ik krijg een soort angstgevoel alsof er elk moment iets vreselijks kan

- ☐ Zeker
- ☐ Heel zeker en vrij erg
- ☐ Ja, maar niet zo erg
- ☐ Een beetje, maar ik maak me er geen zorgen over

43. Helemaal niet

- ☐ Ik kan lachen en de dingen van de vrolijke kant zien
- ☐ Net zoveel als vroeger
- ☐ Niet zo goed als vroeger
- ☐ Beslist niet zoveel als vroeger

44. Helemaal niet

- ☐ Ik maak me vaak ongerust
- ☐ Heel erg vaak
- ☐ Vaak
- ☐ Af en toe maar niet vaak

45. Alleen soms

- ☐ Ik voel me opgewekt
- ☐ Helemaal niet
- ☐ Niet vaak
- ☐ Soms

46. Meestal

- ☐ Ik kan rustig zitten en me ontspannen
- ☐ Zeker
- ☐ Meestal
- ☐ Niet vaak
- ☐ Helemaal niet

47. Ik voel me alsof alles moeizamer gaat

- ☐ Bijna altijd
- ☐ Heel vaak
- ☐ Soms
- ☐ Helemaal niet

48. Ik krijg een soort benauwd, gespannen gevoel in mijn maag

- ☐ Helemaal niet
- ☐ Soms
- ☐ Vrij vaak
- ☐ Heel vaak

49. Ik heb geen interesse meer in mijn uiterlijk

- ☐ Zeker
- ☐ Niet meer zoveel als zou moeten
- ☐ Waarschijnlijk niet zoveel
- ☐ Evenveel interesse als vroeger

50. Ik heb helemaal geen rust en voel dat ik iets te doen moet hebben

- ☐ Heel erg
- ☐ Tamelijk veel
- ☐ Niet erg veel
- ☐ Helemaal niet

51. Ik verheug me van tevoren al op dingen

- ☐ Net zoveel als vroeger
- ☐ Een beetje minder dan vroeger
- ☐ Zeker minder dan vroeger
- ☐ Bijna nooit

52. Ik krijg plotseling gevoelens van panische angst

- ☐ Zeer vaak
- ☐ Tamelijk vaak
- ☐ Niet erg vaak
- ☐ Helemaal niet

53. Ik kan van een goed boek genieten, of van een radio- of televisieprogramma

- ☐ Vaak
- ☐ Soms
- ☐ Niet zelden
- ☐ Heel zelden
- ☐ Helemaal niet

Stel u voor dat er een urinetest bestaat waardoor u geen cystoscopie dient te ondergaan. Een cystoscopie kan wel in uw eigen ziekenhuis plaatsvinden maar het urineonderzoek niet. Als u liever het urineonderzoek wilt laten doen moet u de urine voor het urineonderzoek ZELF naar een ander ziekenhuis brengen. Het wegbrengen van de urine kost u reistijd (de kosten maken niet uit; de verzekering zou de extra reiskosten vergoeden).

80. Hoeveel uren reistijd bent u bereid in totaal (heen en terugreis) te reizen voor een urineonderzoek in plaats van een cystoscopie?

- ☐ 0 uur
- ☐ 1 uur
- ☐ 2 uur
- ☐ 3 uur
- ☐ 4 uur
- ☐ 5-6 uur
- ☐ 7-8 uur
- ☐ 9-10 uur
- ☐ meer dan 10 uur

Na een cystoscopie hoort u direct of uw blaas wel of niet in orde is, maar op de uitslag van urineonderzoek moet u een aantal dagen wachten. Als u dus direct de uitslag wilt hebben, zou u moeten kiezen voor cystoscopie.

81. Hoeveel dagen zou u bereid zijn te wachten op de uitslag van een urineonderzoek?

- ☐ 0 dagen
- ☐ 1 dag
- ☐ 2-3 dagen
- ☐ 4-7 dagen
- ☐ 8-10 dagen
- ☐ 11-14 dagen
- ☐ meer dan 14 dagen

82. Tenslotte; het urineonderzoek kan om technische redenen mislukken. Om met zekerheid te kunnen vaststellen of uw blaas gezond is, zal worden geadviseerd alsnog een cystoscopie te ondergaan (1 op de 20 urinetesten kan mislukken) Zou u het dan erg vinden om alsnog een cystoscopie te ondergaan :

Ja, omdat ……
……
……

Nee, omdat ……
……
……

PART V

PUBLIC HEALTH EFFECTS

CHAPTER 10

Cost-effectiveness of Microsatellite Analysis and cystoscopy

CHAPTER 10

Non-muscle invasive bladder cancer surveillance in which cystoscopy is partly replaced by microsatellite analysis on urine: a cost-effective alternative? (CEFUB-trial)

Esther W. de Bekker-Grob
Madelon N.M. van der Aa
Ellen C. Zwarthoff
Marinus J.C. Eijkemans
Bas W. van Rhijn
Theo H. van der Kwast
Ewout W. Steyerberg

Abstract

To determine how good microsatellite analysis (MA) markers on voided urine samples should be to make a surveillance procedure cost-effective in which cystoscopy is partly replaced by MA for patients with non-muscle invasive urothelial carcinoma (NMI UC). We constructed a semi-Markov model with a time horizon of two years, and a man aged 65 as reference case. Data were used from a randomized trial (including 448 patients with NMI UC from 10 hospitals), and from other data sources. The costs and effects (probability of being in a specific health state) were compared for two surveillance strategies: 1) cystoscopy of the urinary bladder every three months (conventional arm), and 2) semi-automated MA on voided urine samples to identify loss of heterozygosity (LOH) every three months with a control cystoscopy performed at 3, 12 and 24 months (test arm). Various sensitivity analyses were performed to determine the MA sensitivity, MA specificity, and costs of MA-urine for which the test arm was as cost-effective as the conventional arm.

The probability of being without recurrence after two years of surveillances was approximately the same (86.6% conventional arm versus 86.3% test arm) with currently available MA markers (sensitivity of 58% and specificity of 73%). However, the test arm led to higher costs (€4,104 versus €3,433 per capita). The test arm would be as effective and cost the same as the conventional arm if the sensitivity of the currently available MA-markers increased at least to 61%, had a specificity of 73%, and decreased the costs of MA-urine test per follow-up moment from €158 to below €70.Over the course of two years, surveillance in which cystoscopy is partly replaced by currently available urinary microsatellite analysis to reduce patient burden can only provide a cost-effective alternative to the conventional surveillance if the MA urine test would slightly increase its sensitivity and its costs can be reduced.

Introduction

In the Western world bladder cancer is the fourth most common malignancy among men, following prostate, lung, and colon cancers[1]. Histologically, 90-95% of bladder cancers in the western world are urothelial carcinoma (UC). UC not only originate from urothelial lining of the bladder, but they may also occur in pyelum, the ureters or the urethra. Clinically, a distinction is made between non-muscle-invasive (NMI) and muscle-invasive UC. NMI UC are generally treated surgically by a transurethral resection (TUR). Although these NMI UC generally behave rather indolent, their recurrence rate is high (up to 75%). Close monitoring of patients with a NMI UC is warranted since about 10-20% of recurrent tumours may show progression to muscle invasive UC. Muscle invasive UC is a life-threatening disease, since the 5-year survival is about 50%, even after curative surgery (i.e. cystectomy). Surveillance of patients with NMI UC is by regular cystoscopy (in combination with cytologic examination in case of high-risk NMI UC). Although cystoscopy is considered as the reference standard for detection of tumour recurrences, a drawback of the method is its inability to demonstrate recurrences in the upper urinary tract (pyelum, ureter). Moreover, cystoscopy is an invasive procedure, which may be associated with subsequent complaints of anxiety, dysuria and urinary tract infections, adversely influencing the quality of life of the patients[2-4]. It is for these reasons that substantial efforts have been undertaken to design strategies that could reduce the frequency of cystoscopy in patients with NMI UC[5, 6]. One of these strategies is to replace cystoscopy partly by microsatellite analysis (MA) to identify loss of heterozygosity (LOH) in urine samples; literature showed that MA is a highly-sensitive and specific marker for UC diagnosis and its monitoring, especially in patients with low-stage and low-grade tumours[7]. Van der Aa et al.[8] showed in a randomized controlled trial that MA has the ability to detect tumours earlier and to detect tumours in the upper urinary tract, which might be missed by cystoscopy. However, the sensitivity of MA (58%) and specificity of MA (73%) for detecting recurrences in the bladder were both worse than cystoscopy (98% and 88%, respectively)[8].

In the present study, we determined the requirements in terms of sensitivity, specificity, and real costs of microsatellite analysis (MA) markers on voided urine samples to achieve cost-effectiveness of a surveillance procedure of patients with NMI UC whose cystoscopy is partly replaced by MA. We hereto used data from the randomized controlled Cost-effectiveness of Follow-up of urinary Bladder Cancer (CEFUB) trial.

Patients and Methods

Main study

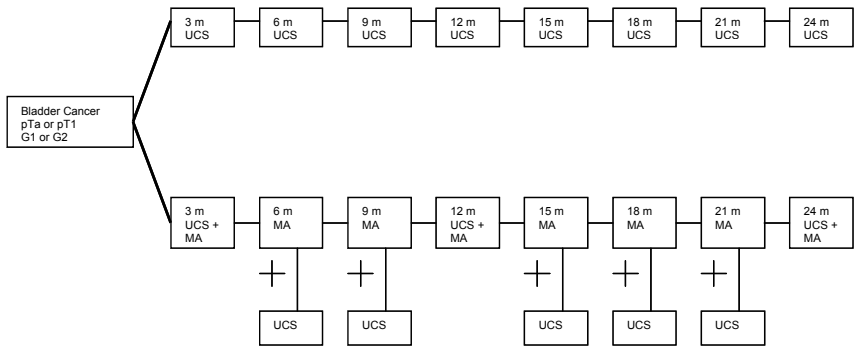
We conducted the randomized controlled CEFUB trial (n=448 patients with NMI UC; recruited by 10 Dutch hospitals; registration ClinicalTrials.gov NCT00126958) to evaluate the efficacy of

MA on voided urine to detect tumour recurrences in the follow-up of patients. Patients were included with a primary or recurrent NMI UC (pTa, pT1, G1 or G2) of the urinary bladder, based on histopathological examination of the surgically removed tumour. Figure 10.1 shows the interventions in the conventional and test arm of the randomized study during two years. The conventional arm consisted of cystoscopy of the urinary bladder every three months. The test arm consisted of semi-automated MA of DNA (for 20 polymorphic microsatellite markers localised 10 chromosomes) extracted from voided urine samples from patients with a NMI UC every three months. A blood sample was taken as a control for the MA of the subsequent urine samples, and was used during the entire study period. A control cystoscopy was performed at 3, 12 and 24 months after TUR, and in case of positive MA outcome. If a tumour recurrence was found by cystoscopy, irrespective of conventional or test arm, it was removed by TUR. In case of progression to muscle-invasive UC or G3 NMI UC after adjuvant intravesical treatment, a cystectomy was considered. If the outcome of MA and cystoscopy were discordant, i.e. MA was positive but no tumour was detected by cystoscopy during two subsequent visits (i.e. a persistently positive MA), an upper urinary tract radio-imaging was performed in order to examine the upper urinary tract (UUT) for the presence of UC. The UUT radio-imaging was not performed immediately in case of positive MA and negative cystoscopy, since previous studies showed that a positive MA test outcome frequently antedates cystoscopically detectable recurrences[8, 9]. In case of suspicion for an UC in the UUT, a unilateral nephroureterectomy was considered.

Decision model

We constructed a semi-Markov model to assess costs and effects (probability of being in a specific health state) of both bladder cancer surveillances (i.e. test arm and conventional

Figure 10.1



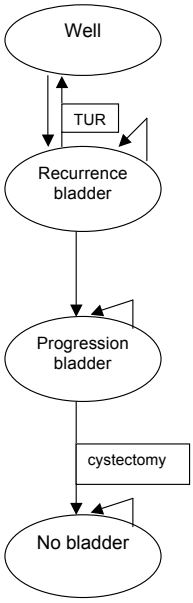
Interventions in the conventional and test arm of the randomised study, in which the plus sign indicates a positive MA test.

arm). The model was implemented in DATA software (Version TreeAge PRO 2007, TreeAge Software, Boston, Massachusetts). Figure 10.2 highlights the core of the surveillance model: monitoring patients with NMI UC can prevent progression to muscle-invasive UC by detecting and treating recurrent tumours first (i.e. it is possible to prevent progression). Appendix 1 shows the underlying conceptual model: missing patients with recurrent tumours can lead to cystectomy and a higher probability of death due to progressive tumours. Poor health states indicate dead, no bladder, progression (i.e. muscle-invasive UC or G3 NMI UC) and recurrent UC, and all other health states indicate 'well'. Noteworthy, after a detected recurrent UC, the patient will go back to the health state 'well' again. Transition probabilities per three months were used to correspond with the moments of follow-up. The reference case was a man aged 65 years, and the time horizon was two years. To retain some memory in the Markov model some health states were constructed with a sub health state. The sub health states indicated whether the MA was positive and the cystoscopy negative in the last visit of surveillance. When this was also the case in the subsequent visit, an IVU was assumed to be performed.

Baseline assumptions

Table 10.1 shows the variables used in our Markov model for insight into the current effects and costs of both strategies. Data from the trial (n=448) were used to determine the three-months probabilities of recurrence (p_{rec}=0.058) and progression after recurrence NMI bladder

Figure 10.2



Core of the model for bladder cancer surveillance: monitoring patients with NMI-UC can prevent progression to muscle-invasive UC by detecting and treating recurrent tumours first (i.e. it is possible to prevent progression). This figure was used for the construction of the Markov model.

cancer (p_{prog}=0.157)[8]. Also, three-months probabilities to undergo a cystoscopy, sensitivity (58%) and specificity (73%) of MA, and sensitivity and specificity of cystoscopy in the bladder (98% and 88% respectively) were determined by our trial data. Detailed, integral cost data of the MA analysis (blood and urine analysis) were obtained from the Department of Pathology of the Erasmus Medical Centre Rotterdam. Cost data of cystoscopy, IVU, TUR, cystectomy, and unilateral nephroureterectomy were obtained from the Department of Finance of the Academic Medical Centre Amsterdam. Unit prices were determined by following the micro-costing method[10], which is based on comprehensive bottom-up analyses and included costs of employment, material, and equipment. A societal perspective was adopted. Costs of one cystoscopy procedure were calculated as €168 per patient (the including personal costs were

Table 10.1
Baseline assumptions Markov-model

Description	SE & SP (%)	Note	Source
Sensitivity MA in bladder	58		Study
Sensitivity MA in UUT	58		Study
Specificity MA in bladder	73		Study
Specificity MA in UUT	73		Study
Sensitivity cystoscopy in bladder	98		Study
Sensitivity cystoscopy in UUT	0		Study
Specificity cystoscopy in bladder	88		Study
Specificity cystoscopy in UUT	100		Study
Sensitivity IVU	60		http://www.guideline.gov
Specificity IVU	100	False positives are not possible	
Description	**3-months probability**	**Note**	**Source**
Dead after progression probability	0.12 0.085	3-months risk in 1st year 3-months risk in 2nd year	Dutch Comprehensive cancer centres Dutch Comprehensive cancer centres
Progression after recurrence probability	0,157		Study
Recurrence probability	0,058	Selection primary tumour to third recurrence	Study
Recurrence probability UUT	0,1	Given there is a recurrence	Dutch Comprehensive cancer centres
Recurrence bladder after nefro-ureterectomy probability	0,12		Raman JD et al (2007, Urology 69(2))
Description	**Costs**		**Source**
MA blood analysis	€ 357		Dep. Of Pathology, Erasmus MC (NL)
MA urine analysis	€ 158		Dep. Of Pathology, Erasmus MC (NL)
Cystoscopy	€ 168		Dep. Of Finance, AMC (NL)
IVU	€ 165		Dep. Of Finance, AMC (NL)
TUR	€ 1,409		Dep. Of Finance, AMC (NL)
Cystectomy	€ 7,997		Dep. Of Finance, AMC (NL)
Nephro-ureterectomy	€ 3,333		Dep. Of Finance, AMC (NL)

relatively low (€63.4 per patient) due to the short diagnostic time and efficient organisation in the participating hospitals). We compared these costs with the determined unit prices of the Department of Finance of Erasmus Medical Centre Rotterdam. The costs were about the same. Confidentially agreements did not allow us to give more detailed information. Costs of a single MA-blood analysis and MA-urine analysis were respectively €357 and €158. MA-blood acted as reference value for MA-urine analysis. Therefore, during two years of surveillance costs of MA-blood analysis were made only once (i.e. all costs were made at the beginning of the surveillance). However, costs of MA-urine analysis were made every moment of follow-up.

In our study we made the following assumptions: 1) progression takes place after recurrence, 2) only one recurrence can take place at the same time, 3) the health states 'recurrence in UUT after nephroureterectomy' does not exist; this prevents a second possible nephroureterectomy in the model, 4) only patients, who had a progression of the tumour at a certain moment, have a higher probability of death than the background mortality, 5) a cystoscopy never reveals a progressive recurrence, which originated in the UUT and invades the bladder, 6) upper urinary tract imaging has a specificity of 100%, 7) in case of progression to muscle invasive UC the next step was a cystectomy, 8) after cystectomy the surveillance stops, and 9) in case of a positive IVU the next step was a nephroureterectomy.

Sensitivity analysis

One-way sensitivity analyses were performed to evaluate the effects of varying costs, recurrence rate, progression rate, sensitivity rates, and specificity rates. All point estimates of these variables were halved or doubled, except the point estimates of the sensitivity and specificity rates which were increased and decreased based on literature[8, 11].

Finally, by performing a two-way sensitivity analysis between sensitivity and specificity of MA, we determined a threshold line at which both surveillance strategies were equally effective. Various combinations of MA sensitivity, MA specificity, and costs of MA-urine were determined in which the test arm was as effective and costs the same as the conventional arm.

Results

Table 10.2a and Table 10.2b show the current costs and effects based on the Markov model for the conventional arm and the test arm respectively for different moments of follow-up. Over the course of two years, total costs of surveillance were lower (€3,433 per capita, €1,283 diagnostic and €2,150 therapeutic costs) for the conventional arm, than for the test arm (€4,104 per capita, €2,347 diagnostic and €1,757 therapeutic costs) (see column 3, stage 24 months). For the conventional arm the cost per capita decreased by every subsequent stage

(column 2), because the probability to be in health states "Dead" (column 4) or "No bladder" (column 5) increased over the time. For the test arm the costs per capita at stage three months were more than twice as high as the conventional arm. At stage three months not only MA urine-analysis took place, but also a MA blood-analysis and a cystoscopy. Stages 12 and 24 months were more expensive than the conventional arm due to the control cystoscopy.

Over the course of two years, there was a probability of 6.1% to lose the bladder in the conventional arm and 7.9% in the test arm (see column 5, stage 24 months). The probability of having a progressive tumour in the bladder was very low, but higher (0.04%) in the test arm compared to the conventional arm (0.01%) (column 7, stage 24 months). The study protocol indicated an IVU and/or CT after two-times discordant outcomes between MA and cystoscopy in subsequent visits in the test arm. In the control arm were additional images of the upper urinary tract executed if clinical performance was suspect. This resulted in a higher detection of UUT carcinomas in the test arm compared to the control arm; 5 UUTca by 52 imaging examinations in the test arm and 2 by 28 examinations in the control arm. However, the test arm detected more tumours because of early suspicion by the urinary test

Table 10.2

A: detailed overview of costs and effects in the conventional arm

Stage	Costs per stage	Cum. costs	Pdead	Pno bladder	Pprog-UUT	Pprog-bladder	Prec-UUT	Prec-bladder	Pwell
months	€	€	%	%	%	%	%	%	%
3	451	451	0,37	0,80	0,09	0,02	0,49	0.09	98,15
6	447	897	0,75	1,61	0,25	0,02	0,89	0.09	96,40
9	440	1337	1,15	2,40	0,44	0,02	1,21	0.09	94,69
12	433	1769	1,57	3,18	0,66	0,02	1,48	0.08	93,01
15	426	2195	1,99	3,94	0,92	0,02	1,70	0.08	91,35
18	419	2615	2,43	4,68	1,19	0,02	1,87	0.08	89,73
21	412	3027	2,89	5,41	1,47	0,02	2,01	0.08	88,13
24	406	3433	3,37	6,12	1,74	0,01	2,12	0.08	86,57

B: Detailed overview of costs and effects in the test arm

Stage	Costs per stage	Cum. costs	Pdead	Pno bladder	Pprog-UUT	Pprog-bladder	Prec-UUT	Prec-bladder	Pwell
months	€	€	%	%	%	%	%	%	%
3	964	964	0,37	0,80	0,09	0,02	0,49	0,09	98,15
6	336	1300	0,75	1,27	0,21	0,36	0,76	1,91	94,75
9	355	1655	1,18	2,05	0,34	0,60	0,98	3,37	91,47
12	713	2367	1,66	3,81	0,46	0,04	1,11	0,11	92,81
15	323	2690	2,06	4,26	0,59	0,35	1,21	1,85	89,68
18	346	3036	2,50	5,02	0,70	0,58	1,27	3,22	86,72
21	366	3402	2,97	6,00	0,79	0,76	1,29	3,93	84,27
24	702	4104	3,45	7,94	0,86	0,04	1,30	0,11	86,29

and detected more UUT carcinomas, hence patients in the test arm were in a slightly worse health state. Therefore, the current conventional and the test arm were more or less equally effective over the course of two years (Figure 10.3). Thus, the Markov model suggested, as expected, that the conventional arm dominated the test arm in terms of cost-effectiveness. The conventional arm had lower costs and a slightly higher number of patients in a good

Table 10.3
Sensitivity analysis on costs and effects for both surveillance arms over the course of two years

	Cum. costs test arm (€)	Cum. costs conv. Arm (€)	Diff. in cum. Costs (test arm minus conv arm) (€)	Prob. of being well in test arm (%)	Prob. of being 'well' in conv arm (%)	Diff. in prob. Of being 'well' (test arm minus conv arm) (€)
Variability						
Recurrence Rate						
0.029	3,629	3,053	576	91.48	91.69	-0,21
0.116	4,954	4,135	819	77.04	77.08	-0,04
Progression Rate						
0.079	3,905	3,276	629	90.17	89.52	0.65 **
0.314	4,440	3,731	709	79.65	80.94	-1,29
Costs cystoscopy (€)						
84	3,745	2,792	953	86.29	89.57	-3,28
336	4,823	4,716	107	86.29	86.57	-0,28
Costs MA blood (€)						
179	3,926	3,433	493	86.29	89.57	-3,28
714	4,460	3,433	1027	86.29	86.57	-0,28
Costs MA urine (€)						
79	3,502	3,433	69	86.29	89.57	-3,28
316	5,308	3,433	1875	86.29	86.57	-0,28
Sens rate MA						
0.29	4,079	3,433	646	83.87	86.57	-2,70
0.70	4,111	3,433	678	87.27	86.57	0,70 **
Spec rate MA						
0.69	4,181	3,433	748	86.30	86.57	-0,27
0.76	1,048	3,433	615	86.28	86.57	-0,29
Sens rate cystoscopy						
0.84	4,093	3,432	661	84.63	85.31	-0,68
0.98	4,104	3,433	671	86.29	86.57	-0,28
Spec rate cystoscopy						
0.79	4,583	4,327	256	86.29	86.57	-0,28
0.98	3,572	2,439	1133	86.29	86.57	-0,28

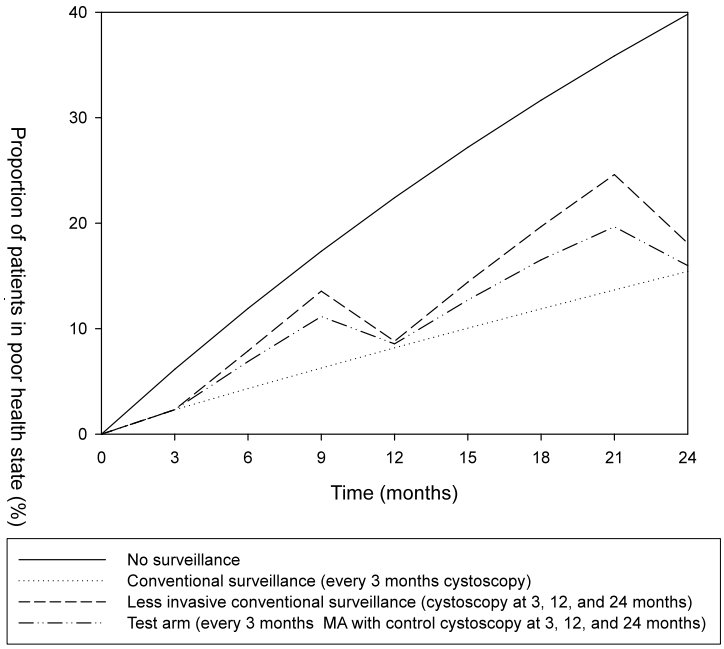
* Test arm is more expensive than conventional arm over the course of two years

** Test arm is more effective than conventional arm over the course of two years

health state (86.6% versus 86.3%; see Tables 10.2a and 10.2b respectively, column 10, stage 24 months). Figure 10.3 also showed that 1) if patients would only get MA without a control cystoscopy at stage 3, 12, and 24 months, the probability of being in a poor health state would increase steeply, and 2) if patients would only get a cystoscopy at stage 3, 12, and 24 months without MA, a lower number of patients would be in a good health state compared with the test arm.

Table 10.3 shows various one-way sensitivity analyses on costs and effects for both surveillance arms over the course of two years. Changing the variables over plausible ranges did not make the test arm cheaper than the conventional arm. Only if the progression rate was halved

Figure 10.3



Probability (%) of being in a poor health state in either surveillance strategy (conventional or test arm) for different moments of follow-up. Poor health states indicate dead, no bladder, progression and recurrence UC.

Table 10.4

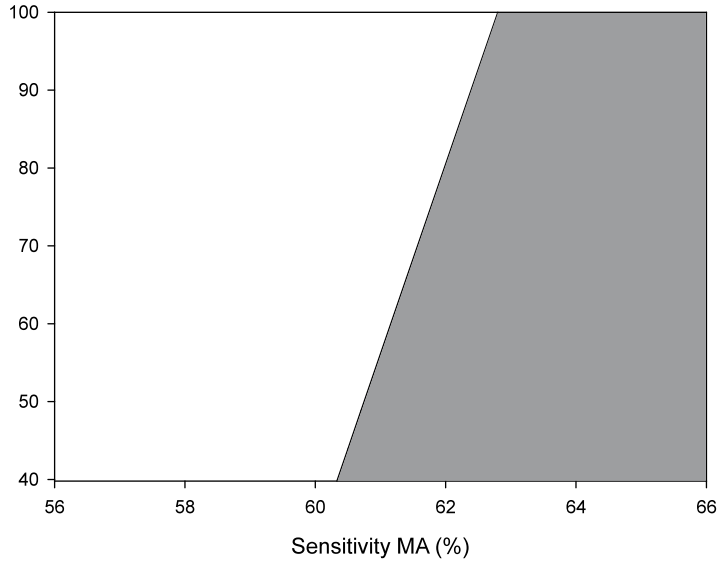
Combinations of MA sensitivity, MA specificity, and costs of MA-urine in which the test arm is as effective and cost the same as the conventional arm

Sensitivity MA	Specificity MA	Costs of MA-urine
(%)	(%)	(€)
60,5	47	0,0
60,7	53	17,0
61,4	73	69,6
62,8	100	130,5

(i.e., 0.079) the probability of being "Well" (i.e., being without a recurrence or progressive UC) was larger in the test arm than the conventional arm (90.17% vs 89.52% respectively) over the course of two years. This was also the case, if the sensitivity of MA was increased to 70% (i.e., probability of being "Well" was 87.27% in the test arm vs. 86.57% in the conventional arm). Obviously, changing the costs did not influence the health effect side.

Figure 10.4 shows that the test arm was equally effective as the conventional arm, if the MA had a sensitivity of 60.3% in combination with a specificity of 39.8%, or if the MA had a sensitivity of 62.8% in combination with a specificity of 100%, or all linear combinations of MA sensitivity and specificity in-between. Table 10.4 shows that, for instance, if the sensitivity would increase to 61%, the specificity of MA remains at 73%, and the costs of MA-urine will decrease to €70, than the test arm is as effective and cost the same as the conventional arm.

Figure 10.4



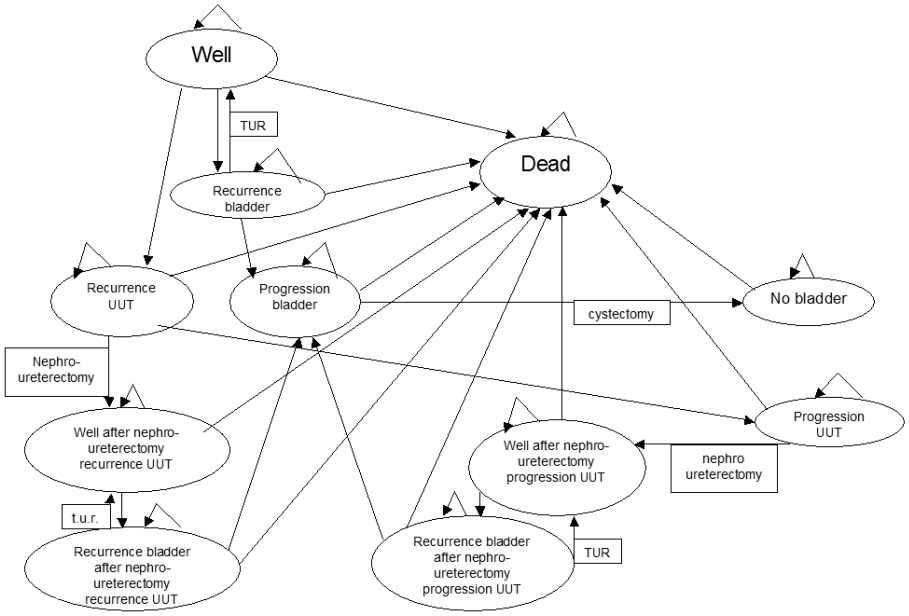
Threshold analysis for surveillance strategies. Sensitivity and specificity combinations in the *white* area indicate that the conventional arm is more effective; sensitivity and specificity combinations in the *grey* area means that the test arm is more effective.

* = current assumption for MA test (MA sensitivity 58%, MA specificity 73%)

x = if MA-urine test has a sensitivity of 60.5%, a specificity of 47%, and will be free of charge, the test arm will be as effective and cost the same as the conventional arm

0 = if MA-urine test has a sensitivity of 62.8% and a specificity of 100%, and will cost €130.5, the test arm will be as effective and cost the same as the conventional arm

Figure 10.5



Discussion

Currently available MA markers are not sensitive enough (sensitivity of 58%) or specific (specificity of 73%) to use as a replacement of cystoscopy in the surveillances of bladder cancer. The probability of being without recurrence after two years of surveillances was approximately the same for both surveillance strategies (86.6% conventional arm vs 86.3% test arm), but the test arm led to higher costs (€4,104 versus €3,433 per capita). The conventional arm hence dominated the test arm (less costs, similar effects). However, the test arm will be as effective and cost the same as the conventional arm if the sensitivity of the current available MA-markers increases to at least 61%, the specificity of MA remains at 73% and the costs of MA-urine test per follow-up moment would decrease from €158 to below €70.

Lotan and Roehrborn[12] used a decision tree to evaluate the cost-effectiveness of substituting cystoscopy by other bladder tumour markers than MA markers. They showed that using a urine based tumour marker alternating with cystoscopy is cost-effective for a wide range of marker sensitivities, specificities and costs, a 20% to 80% yearly recurrence and a 2% to 40% yearly progression rate. Although the sensitivity and specificity of their tumour markers and our MA markers were almost the same as in our study (respectively, 50% versus 58%, and 70% versus 73%), the costs of diagnostic and therapeutic interventions used in both studies differed, especially with respect to the costs of the tumour markers. Ignoring the inflation rate, the costs of the tumour markers were assumed as $50[12], in contrast to €158 for our

MA urine analysis and €357 for the one-time MA blood analysis. It is plausible that genetic analysis (i.e. MA markers) are more expensive than urine sediment diagnosis. However, for a fair comparison of costs we should use the same costing method. The costs in our study were based on unit prices determined by following the micro-costing method[10], while the costs in Lotan and Roehrborn[12] were according to the listing of the manufacturers. Another important difference between both studies is that in our study at stage three, 12 and 24 months a control cystoscopy took place in addition to MA urine-analysis, which increased the costs.

Our Markov-model did not explicitly account for the burden (i.e. quality of life; e.g. pain, infection rate) associated with cystoscopy. Cystoscopy would take place 7.6 times in the conventional arm and just 4.3 times in the test arm over the course of two years. However, we assumed that this difference is not enough to compensate for the more frequent cystectomy or greater risk of dying from cancer. Further, in the test arm at stage three, 12 and 24 months MA urine-analysis took place in addition to cystoscopy. Cystoscopy has by definition a high sensitivity. Therefore, at these stages the MA-urine-analyses might be superfluous and its costs can be saved here; i.e. in practice urologists should use MA as a substitute rather than as an adjunct for bladder cancer detection and follow-up. Another point is that MA-blood analysis was required to provide reference values for MA-urine analyses. The costs of MA-blood analysis are made only once, at the start of surveillance. Over the course of e.g. ten years instead of two years of surveillance, there will hence be less difference in total costs between both arms.

Our study had some further limitations, primarily related to various assumptions that had to be made in the model to make the model manageable. For example, we assumed that progression only takes place after recurrence. However, it is conceivable that progression occurs directly from the health state "Well". Based on data from the study, we may assume that MA is more sensitive for progression than for recurrence. In all, this would make the cost-effectiveness for MA worse. Finally, it is not immediately clear how to exactly translate our cost estimates in other countries or clinical practice routines. The costs may vary from one area to another in Europe and in the United States, and the panel of tested markers can be improved. Further studies in a larger international population in a prospective manner are therefore necessary.

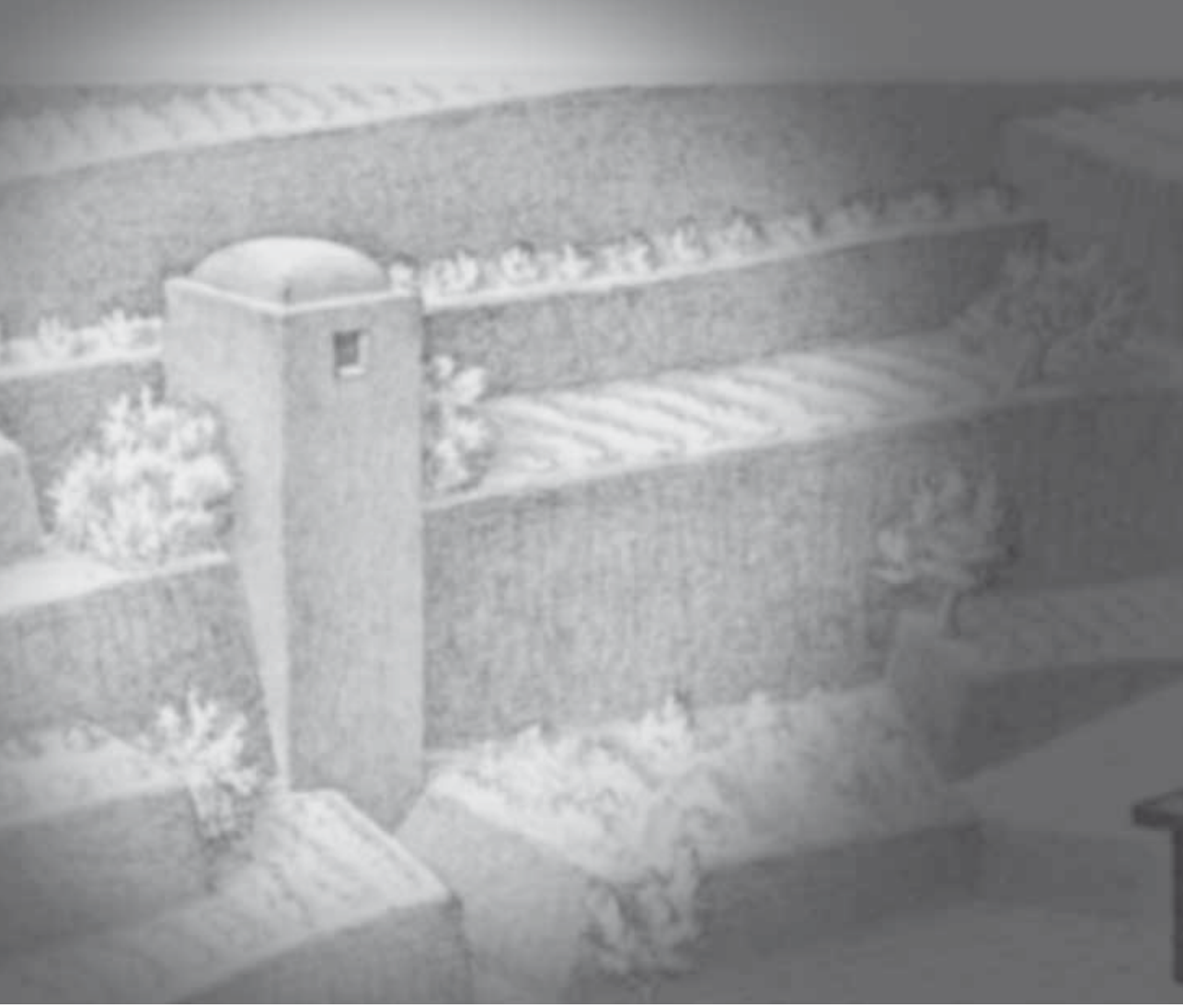
Some recommendations can be made. The costs of MA (blood and urine analysis) were relatively high compared to the costs of cystoscopy. The large difference in cost is caused by material as well as personal costs. Further automation of MA testing might lead to mass production and cost reduction. Research is needed to find out how much costs per capita will decrease due to automation. According to the model, the current MA-markers were not sensitive enough in comparison with cystoscopy. Better MA-markers should be found to obtain better cost-effectiveness. Especially, sensitive markers are of interests that do not require comparison to a blood sample of the patient for reference values. Noteworthy, based

on data from the study it is conceivable that awareness of a positive MA outcome may have prompted a higher detection rate of UC recurrences by cystoscopy. Indeed, separate analysis of the patients of the MA arm showed an improved cross-sectional sensitivity (70%) of the MA test[8]. Recent developments suggest other DNA-based tests as alternatives, such as detection of *FGFR3* mutations (present in the majority of NMI UC) and assays for detection of tumour-associated methylation of genes of interest[13-17], since these tests are cheaper than MA regarding material as well as personnel costs.

In conclusion, over the course of two years, surveillance in which cystoscopy is partly replaced by currently available urinary microsatellite analysis to reduce patient burden can only provide a cost-effective alternative to the conventional surveillance if the MA urine test would slightly increase its sensitivity and its costs can be reduced.

References

1. Kirkali Z, Chan T, Manoharan M, Algaba F, Busch C, Cheng L, et al. Bladder cancer: epidemiology, staging and grading, and diagnosis. Urology. 2005 Dec;66(6 Suppl 1):4-34.
2. Almallah YZ, Rennie CD, Stone J, Lancashire MJ. Urinary tract infection and patient satisfaction after flexible cystoscopy and urodynamic evaluation. Urology. 2000 Jul;56(1):37-9.
3. Denholm SW, Conn IG, Newsam JE, Chisholm GD. Morbidity following cystoscopy: comparison of flexible and rigid techniques. Br J Urol. 1990 Aug;66(2):152-4.
4. Clark KR, Higgs MJ. Urinary infection following out-patient flexible cystoscopy. Br J Urol. 1990 Nov;66(5):503-5.
5. Ross JS, Cohen MB. Ancillary methods for the detection of recurrent urothelial neoplasia. Cancer. 2000 Apr 25;90(2):75-86.
6. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. Pharmacoeconomics. 2003;21(18):1315-30.
7. Bartoletti R, Dal Canto M, Cai T, Piazzini M, Travaglini F, Gavazzi A, et al. Early diagnosis and monitoring of superficial transitional cell carcinoma by microsatellite analysis on urine sediment. Oncol Rep. 2005 Mar;13(3):531-7.
8. van der Aa MN, Zwarthoff EC, Steyerberg EW, Boogaard MW, Nijsen Y, van der Keur KA, et al. Microsatellite Analysis of Voided-Urine Samples for Surveillence of Low-Grade Non-Muscle-Invasive Urothelial Carcinoma: Feasibility and Clinical Utility in a Prospective Multicenter Study (Cost-Effectiveness of Follow-Up of Urinary Bladder Cancer Trial [CEFUB]). Eur Urol. 2008 May 15.
9. Amira N, Mourah S, Rozet F, Teillac P, Fiet J, Aubin P, et al. Non-invasive molecular detection of bladder cancer recurrence. Int J Cancer. 2002 Sep 20;101(3):293-7.
10. Gold MR, Siegel JE, Russel LB, Weinstein MC. Cost-effectiveness in Healt and Medicine. New York: Oxford University Press 1996.
11. Grossman HB, Soloway M, Messing E, Katz G, Stein B, Kassabian V, et al. Surveillance for recurrent bladder cancer using a point-of-care proteomic assay. Jama. 2006 Jan 18;295(3):299-305.
12. Lotan Y, Roehrborn CG. Cost-effectiveness of a modified care protocol substituting bladder tumor markers for cystoscopy for the followup of patients with transitional cell carcinoma of the bladder: a decision analytical approach. J Urol. 2002 Jan;167(1):75-9.
13. Dulaimi E, Uzzo RG, Greenberg RE, Al-Saleem T, Cairns P. Detection of bladder cancer in urine by a tumor suppressor gene hypermethylation panel. Clin Cancer Res. 2004 Mar 15;10(6):1887-93.
14. Rieger-Christ KM, Mourtzinos A, Lee PJ, Zagha RM, Cain J, Silverman M, et al. Identification of fibroblast growth factor receptor 3 mutations in urine sediment DNA samples complements cytology in bladder tumor detection. Cancer. 2003 Aug 15;98(4):737-44.
15. Miyake M, Sugano K, Kawashima K, Ichikawa H, Hirabayashi K, Kodama T, et al. Sensitive detection of FGFR3 mutations in bladder cancer and urine sediments by peptide nucleic acid-mediated real-time PCR clamping. Biochem Biophys Res Commun. 2007 Nov 3;362(4):865-71.
16. van Oers JM, Lurkin I, van Exsel AJ, Nijsen Y, van Rhijn BW, van der Aa MN, et al. A simple and fast method for the simultaneous detection of nine fibroblast growth factor receptor 3 mutations in bladder cancer and voided urine. Clin Cancer Res. 2005 Nov 1;11(21):7743-8.
17. van Rhijn BW, Lurkin I, Chopin DK, Kirkels WJ, Thiery JP, van der Kwast Th, et al. Combined microsatellite and FGFR3 mutation analysis enables a highly sensitive detection of urothelial cell carcinoma in voided urine. Clin Cancer Res. 2003 Jan;9(1):257-63.



PART VI

EPILOGUE

CHAPTER 11

General discussion, future perspectives & concluding remarks

CHAPTER 12

Summary and Samenvatting

CHAPTER 13

Acknowledgements

CHAPTER 11

General Discussion, Future Perspectives & Concluding Remarks

Perspectives on management of patients with non-muscle invasive bladder cancer

The medical disciplines involved in the management of patients diagnosed with a low-grade, low stage bladder cancer may have a different perspective on how to define an optimal treatment and surveillance strategy. The clinician/urologists' point of view on the disease may differ from that of the pathologist, and both their views will not necessarily coincide with the patient's perspective, or with the societal (public health) perspective. An urologist will require an accurate and unequivocal clinical diagnosis, which can be translated directly into the most suitable treatment and surveillance regimen. The urologist primarily wants to limit the risk of treatment failure as much as possible and secondly he/she wants to prevent over-treatment. A pathologist may understand this view but wants to emphasize that the microscopic and molecular aspects of bladder cancer may be highly variable. The histological features cover a spectrum of diseases rather than distinct entities. This results in a subtler and more nuanced separation of bladder cancers, which may not necessarily be of clinical relevance. A patient diagnosed with bladder cancer primarily wants to know what it means for his/her future, and he/she desires the best possible treatment and the most rigorous surveillance scheme, often irrespective of burden, in order to have the best survival options. From the societal perspective the interests of the bladder cancer patient have to be balanced against those of other health care 'clients' based on a cost-effectiveness analysis of treatment modalities and surveillance (which also includes quality of life aspects) in order to have a maximum bang for the buck. This thesis tries to reflect the above-mentioned perspectives particularly in an attempt to define the requirements for an optimal surveillance and treatment strategy for these patients.

Bladder cancer is a common disease with high prevalence as a consequence of its chronicity and represents the fifth most expensive cancer to treat, while incurring the highest cost per patient of all cancers[1]. Therefore it is important to obtain comprehensive criteria to define optimal management in surveillance and therapeutic options in patients with bladder cancer. The rationale for a tight surveillance of patients with low-grade low stage bladder cancer is to prevent progression by early interception of recurrences, prior to progressive growth to a potentially life-threatening disease. However, it is also acknowledged that such surveillance regimens are opinion-based and potentially overshoot or undershoot the optimal regimen depending on individual risk. An American study showed that the adherence to clinical guidelines on management of bladder cancer is only 60%[2].

The current surveillance regimen in bladder cancer represents several challenges in clinical management, which can ultimately result in the proposal of individualised tailor-made schemes using alternating invasive (cystoscopy) and alternative non-invasive methods. A

prerequisite for the successful introduction of alternative methods for diagnosis and surveillance is that they must be at least as reliable and feasible in routine clinical practice as the current (cystoscopy) practice. It is commonly held that cystoscopy is both a costly as well as a burdensome procedure for the patient due to its invasive nature. This thesis addressed these two assumptions.

Alternative surveillance schemes may aim at the reduction of cystoscopy frequency. This may be achieved by two mutually non-exclusive strategies: 1) the reliable identification of a subset of patients with NMI UC with a low frequency of recurrence and progression using clinico-pathologic parameters as well as molecular *tissue* biomarkers (addressed in **Part II** of the thesis) and 2) establishment of novel *urine* tests with a sensitivity and specificity that can match cystoscopy (**Part III**).

A major part of this thesis (**Part III-V**) covers the results of a randomized clinical trial on the cost-effectiveness of follow-up of patients with low-grade non-muscle invasive (NMI) bladder cancer (CEFUB trial). The major objective of this trial was to compare a conventional follow-up scheme with cystoscopy only to a scheme including the substitution of a number of cystoscopies by a genetic urine test (i.e. microsatellite analysis or MA to detect genetic alterations) on voided urine specimens. The primary endpoint of the trial was to demonstrate more recurrences in the intervention arm than in the conventional arm (**Part III**). Other important outcome parameters were the difference in progression to higher stage bladder cancer and the identification of upper urinary tract cancers in both trial arms. Further, we studied quality of life aspects (**Part IV)** and costs of the conventional and alternative surveillance scheme (**Part V**).

How to predict bladder cancer behaviour by tissue biomarkers?

Can we predict tumour behaviour (recurrence, progression) and identify subsets of patients by clinical, pathological and molecular features, which characterise clinically distinct behaviour and require specific treatment and surveillance schemes? Studies offering risk-group profiling with nomograms and scoring tables using conventional clinico-pathologic parameters have been put forward to offer probability numbers for recurrence and progression to muscle invasive (≥pT2) bladder cancer. These different studies may be difficult to compare, because the comparability is complicated by the use of different definitions for the common outcome parameters "recurrence" and "progression".

Recurrence and progression

Recurrence may be defined as time to first recurrence, as recurrences in the first year after diagnosis, as recurrences after a negative 3-months control cystoscopy or as number of

recurrences per year (recurrence rate). Similarly, for the term "progression" a number of definitions are in use, although the most common definition is based on "stage" rather than "grade" progression. The issue on how to define "progression" arises from the difference in clinical and pathological perspective. From the clinical perspective, progression is the result of the clinical behaviour of the tumour showing any increase in risk category that can lead to an 'irreversible' treatment decision like e.g. cystectomy. Until curative therapy is achieved, a patient is diagnosed with progressive recurrence if a formerly detected single or multiple NMI UC (pTa, pT1, PUNLMP, low-grade carcinoma) develops either into muscle invasive UC (≥ pT2, high-grade) or presents with CIS, or the patient becomes a non-responder to intravesical therapy or a patient is treated with cystectomy due to disease regardless of the pathological diagnosis. We prefer to define pathological progression of bladder cancer as a recurrence with any histopathologically higher stage (stage progression) or any higher grade (grade progression) as compared to the highest bladder cancer stage/grade in the patient's history. Hence a patient can have a clinical progression without proven pathological progression.

Prognostic factors

Meta-analyses of conventional clinico-pathologic features

We performed a meta-analysis to identify the most potent prognostic factors for time to first recurrence and for pathological stage progression based on data obtained from 14 studies of larger bladder cancer patient series (**chapter 3**) published between 1980 and 2004. Our meta-analysis on disease recurrence was based on 11 publications; covering 5,021 patients. Data on progression were based on just 4 publications; covering 3,313 patients. The latter was caused by differences in definition of progression or unavailability of progression data in the other papers.

Our meta-analysis clearly showed that the prognostic value of conventional clinico-pathologic parameters was of limited value in predicting bladder cancer recurrences. Relative risks were on average 2. The strongest univariable risk factors for recurrence were a residual bladder cancer at the 3-month control cystoscopy (RR 2.2), a history of bladder cancer recurrences (RR 1.5) and trigone involvement (RR 1.5). We concluded that it is not possible to reliably predict time to recurrence with the conventional characteristics. These findings are largely in line with the outcome of another large meta-analysis on a total of 2,596 patients with non muscle invasive bladder cancer who participated in a series of randomized phase III EORTC studies[3]. Both studies employed the same definitions of recurrence and progression. Main prognosticators for recurrence in the EORTC meta-analysis were a history of prior recurrences, multifocality and tumour size with hazard ratios in multivariate analysis ranging from 1.35-1.56. Since the latter meta-analysis did not include residual tumour at the 3-month control cystoscopy as a parameter, this specific feature demonstrated in our meta-analysis

could not be further confirmed. The importance of the recurrence pattern, and particularly the presence of residual carcinoma at the 3-month control cystoscopy for prediction of future recurrences was also emphasized in the earlier study by Fitzpatrick et al[4].

For tumour progression our meta-analysis demonstrated that both tumour grade (using WHO 1973) and presence of (secondary) carcinoma in situ were strong prognostic factors for progression (RR 6.7 and 4.4 respectively), while multiplicity, stage (pTa versus pT1) and tumour size had an intermediate effect (RR between 1.7 and 2.2). In the EORTC meta-analysis the main prognosticators for progression were presence of carcinoma in situ (HR 3.41), (WHO 1973) grade (2.67) and pathological stage (pTa versus pT1, HR 2.19). The data indicate that particularly patients with a pT1 WHO 1973 grade 3 cancer and with secondary carcinoma in situ do poorly. The one and 5-year probability of progression to muscle invasive (pT2) carcinoma were, however with wide confidence intervals, 29% (CI 10-24%) and 45% (CI 35-55%), respectively[3]. It is clear that additional prognosticators especially regarding pT1G3 UC would be of great benefit for clinicians. Treatment decisions in this subset of tumours often have a major impact on the patient's quality of life.

It should be noted that both meta-analyses discussed above are using the WHO 1973 classification system of bladder cancers for the grading of these cancers. In an attempt to improve the prognostication of recurrences and of progression of bladder cancers the most recent WHO 2004 classification of bladder cancers introduced a new grading system of bladder tumours with the intent to distinguish more specifically the cancers with a low risk of recurrence and low risk of progression from those with a higher risk.

WHO 1973 and WHO 2004

In **chapter 4** we evaluated in tumour tissues derived from 221 patients with NMI UC the prognostic value of both the WHO 1973 and 2004 grading system together with the prognostic impact of three tissue biomarkers, CK-20, KI-67 and the *FGFR3* gene mutation. We showed that both the WHO 1973 and 2004 grading systems contribute independent valuable information for prognosis of pathological stage progression in NMI UC. Most importantly, the WHO 2004 high-grade NMI UC now represents - by definition - a more heterogeneous group than the former WHO 1973 grade 3 NMI UC, and our data imply that its prognostic impact may be improved either by adding WHO 1973 grading information (grade 2 versus grade 3) or by adding FGFR3 mutation status. The FGFR3 mutation status was able to differentiate between those who progress and those who do not both in the total patient series and also selectively in the subset of high-grade carcinomas and the subset of pT1 carcinomas. From a molecular perspective, the separateness of mutant and wild type FGFR3 bladder cancers was demonstrated by clearly distinct expression profiles of these tumours[5]. Strikingly, in a much earlier study Holmang et al showed that the WHO 2004 high-grade cancers, comprising by

definition all WHO 1973 grade 3 cancers and a subset of the WHO 1973 grade 2 cancers, can be separated in those more prone to progression and those who are not on the basis of DNA ploidy status[6].

FGFR3 and other biomarkers

Another approach to improve the prognostics of bladder cancer is the identification of molecular markers, which – preferably independent from conventional prognosticators – would help to identify patient at low and at high risk for recurrence and progression. Similar to several studies from our group, one Spanish group also studied the prognostic impact of FGFR3 mutation status in a large series of 772 patients with NMI UC. In their large series, FGFR3 mutation status was only a risk factor for progression in univariable analysis if all patients were considered[7]. In the subset of their patients with pT1 G3 bladder cancer FGFR3 mutation analysis was not able to distinguish the progressors from the non-progressors. To explain the difference in outcome between the two studies it should be noted that 1) the Spanish population comprises a much higher proportion of male patients (7:1) as compared to the Dutch and German population (3:1), 2) the lower frequency of mutant FGFR3 detected in the Spanish study (50%) as compared to our series (62%), 3) the difference in molecular technology used to detect FGFR3 gene mutations, 4) a different definition of "progression" utilized in both studies and 5) the difference in frequency of "progression" in the Spanish series (6.3%) and Dutch series (9%) and 5) the use of the WHO 1973 grading system in the Spanish series and both the WHO 1973 and 2004 grading system in our series.

Similar to most other studies we did not find any prognostic tissue biomarker to predict bladder cancer recurrences. In another large multicenter study on 404 tumours it was also not possible to reproduce the prognostic value of a previously reported gene expression signature for recurrences[8]. In contrast, the previously mentioned Spanish study reported that the presence of the *FGFR3* gene mutation in the subset of patients with a pTaG1 bladder cancer would predict a significantly increased risk of recurrence (HR 2.12). A recent case-control study on primary papillary urothelial neoplasms of unknown malignant potential (a subset of grade 1 WHO 1973 non-invasive bladder cancers) suggested that those without subsequent recurrence could be distinguished on the basis of a high expression of the FGFR3 protein in combination with a normal CK-20 expression pattern[9]. Prospective studies are needed to verify this potentially interesting finding. An alternative approach to identify biomarkers on tissue specimens for recurrence is the analysis of the morphologically normal urothelium in patients with (a history of) bladder cancer. Using this novel approach, Lopez-Beltran et al[10] reported that identification of loss of heterozygosity of DBC1 (deleted in bladder cancer 1 locus) on chromosome 9 in the normal bladder biopsies of a small series of 49 patients with PUN-LMP or low-grade bladder cancer was a strong marker for recurrence, while its presence in the tumour was not of prognostic value.

pT1 bladder cancer

For the group of pT1 bladder cancers some pathologists have proposed substaging on the basis of the depth of tumour invasion relative to the muscularis mucosae and the vascular plexus. Although promising, the WHO 2004 classification of bladder tumours decided not to include this substaging system. Difficulties encountered in the substaging of pT1 bladder cancers according to the depth of invasion are related to the morphologically variable muscularis mucosae and the vascular plexus[11] and consequently to the substantial interobserver variation in substaging among pathologists[12-14].

In **chapter 5** we present a novel approach to the substaging of pT1 bladder cancers based on the extent of invasion. Tumours that show invasion of the subepithelial stroma by a single focus extending less than a maximum length of 0.5 mm (pT1mic) showed better prognosis regarding progression as compared to tumours with multiple invasive foci and/or an extensively (>0.5mm) infiltrating front (pT1ext). The characteristic that activating FGFR3 gene mutations are associated with favourable disease course was again confirmed in this series, as FGFR3 gene mutations were commonly observed in pT1mic and rarely in pT1ext, adding to the biological significance of this morphological distinction. The findings may also suggest that mutant FGFR3 pT1mic and wild type FGFR3 pT1ext do represent separate pathways of bladder carcinogenesis associated with a clinically distinct behaviour. To our opinion, this simple new sub-staging system for pT1 UC is a very promising tool. Recently, its value could be confirmed in a larger series of about 130 pT1 bladder cancers (personal communication: Van der Kwast and Van Rhijn; 2008).

Can we improve surveillance in bladder cancer?

Randomized Clinical Trial

In a final attempt to validate previous smaller studies showing the potential value of microsatellite analysis (MA) on voided urine samples as a safe alternative for cystoscopy during surveillance of patients with low-grade NMI bladder cancer we designed a randomized clinical trial. In one arm the patients were followed by 3-monthly cystoscopy after diagnosis of primary or recurrent NMI UC, and in the other (intervention) arm a number of cystoscopies was replaced by MA testing on voided urine samples. As target population we selected patients with low-grade (WHO 1973 grade 1 and 2), low stage (pTa, pT1) bladder cancer, excluding patients with a history high-grade (WHO 1973 grade 3) cancer and with carcinoma in situ. The latter patients were not considered good candidates for this trial, since they are by definition at high risk for both recurrence and progression, and clinicians would not consider it ethically to omit a number of cystoscopies in these patients.

Microsatellite Analysis (MA-Test) on voided urine

We reported an overall sensitivity of 58% and a specificity of 73% with a PPV of 20%. These figures are lower than the previously reported sensitivity (82%) and specificity (89%)[15]. An explanation is that the latter figures are derived from studies that also included high-grade NMI UC patients. A prospective large size multicenter trial like ours is challenging to perform because of the technical and logistic difficulties. This was exemplified by the observation that about 20% of the urine samples were inadequate for MA because their DNA content was too low, probably due to degradation during prolonged storage and transportation. Moreover the compliance to an intensive prospective study protocol is subject to the readiness of both patients and urologists.

In a longitudinal analysis of our randomized trial results, the 2-yr risk to develop a recurrence reached 83% if MA outcome was persistently positive and 22% when MA was persistently negative. Consecutive positive MA results are thus a strong predictor for future recurrences, but sensitivity of the test still needs to be improved. Our study indicated that this could be achieved by, for example, patient selection and by testing of additional genetic markers in urine samples. As to patient selection we found that MA misses more recurrences in non-smoking patients and in females. Surveillance by MA could be especially valuable for smoking patients with an FGFR3 wild type tumour. Besides we noted that the presence of a FGFR3 mutation in the tumour at the time of enrolment in the trial was negatively correlated with the detection of recurrences using MA. The latter finding can easily be explained, since FGFR3 mutant bladder cancers are genetically more stable, with less LOH. For this reason an obvious additional genetic marker to be tested on voided urine samples could be the FGFR3 mutation itself. As mentioned earlier the *FGFR3* gene mutation is common in low stage and grade NMI UC (up to 75%) and its high frequency in NMI -UC may provide an additional marker. In combination with MA or other assays this biomarker could raise the sensitivity of the test to a clinically more acceptable level for the surveillance of patients with NMI UC. A preliminary study of our laboratory on a small series of patients reported the enhanced sensitivity of a combination of MA and FGFR3 mutation analysis on voided urine samples of NMI UC patients[16]. Van Oers et al reported recently the development of a high-throughput method for FGFR3 mutation analysis on both tumour and urine DNA samples[17]. This easy-to-use and relatively cheap assay can be used in every laboratory with access to a PCR machine and sequencer (T. Zuiverloon, personal communication). The main advantage is that the most common mutations in *FGFR3* can be detected simultaneously in a single assay. A prospective multicenter trial of urinary analysis of FGFR3 combined with MA in the surveillance of patients with NMI UC is currently under way (T. Zuiverloon).

Since the start of the trial two additional papers were published on the application of MA on voided urine samples for the surveillance of patients with NMI UC[18, 19]. The former paper

on 47 patients, including 8 high-grade cancers reports a sensitivity of 92% for detection of recurrences after a median follow-up of 33 months. Interestingly, they mentioned that 75% of the recurrences were detectable between 1 and 9 months by MA prior to the cystoscopic detection of the recurrence. In the second paper based on a prospective series of 91 patients a sensitivity of about 60% (within the range of the value found in our study) was reported, but they noted a higher specificity (93% for recurrences). Again, their higher sensitivity and specificity as compared to our study may be the consequence of the inclusion of patients with high-grade cancers in their study. Notably, just like in our study (**chapter 6**), one case of high-grade UC remained undetected. In their retrospective analysis they noted an improvement of the sensitivity by combining cytology and MA[19].

Another test, based on the detection of genetic abnormalities in cells shed in urine, using fluorescence in situ hybridisation has been marketed for several years, that is the FDA approved UroVysion FISH test. Although the methodology of this test is different from the MA test, it also relies on the detection of genetic changes in voided urine samples. In a very recent paper on 250 patients, including patients with a high-grade UC, using UroVysion on cytological specimens of voided urine the predictive rather than diagnostic capacity of this test was emphasized[20]. The sensitivity of this test for detection of a recurrence was about 60%. In this study 3 high-grade, high stage cancers were missed by the FISH test. The same authors reported that in 35 of 56 (62.5%) patients with a positive UroVysion test outcome the cystoscopy became positive at a later time during a total follow-up period of 29 months. In their population of bladder cancer patients 27% would have a positive test outcome without a concomitant cystoscopically detectable recurrence. The findings of our randomized CEFUB trial are thus very much in line with 1) the somewhat lower than expected sensitivity of these genetic tests (MA and UroVysion-FISH) during follow-up of low-grade low stage bladder cancer and 2) the gradually evolving concept that genetic changes either detectable by MA test or by FISH analysis often precede the actual cystoscopically detectable recurrence several months later. As shown in our trial, the latter results into a very low positive predictive value of the MA test (20%). It would suggest that at the time that no detectable bladder cancer is present, genetically abnormal cells continue to be shed in the urine, as a very early manifestation of a future recurrence. This matches very well with the LOH study on urinary bladder tissue biopsies with normal morphology, revealing a good correlation of LOH at the DBC1 locus on chromosome 9 and recurrence[21].

Based on the relatively low overall sensitivity (58%) and specificity (73%) found by cross-sectional analysis in our series (**chapter 6**) we concluded that urine tests on voided urine samples based on the detection of genetic changes (i.e. the MA test, but this holds also true for UroVysion FISH) cannot be recommended for implementation in the surveillance of patients with non muscle invasive bladder cancer.

Diagnostic Review Bias

In our randomized trial we demonstrated a significantly increased number of recurrences detected in the intervention (MA) arm as compared to the control (conventional cystoscopy) arm. This could have indicated that the intervention was very effective, but this conclusion does not add up with the observed low sensitivity, specificity and PPV of the MA test. A potential limitation of our study is that our main analysis was based on the combined data of the two trial arms, one of which comprised the unblinding of the urologist for the MA outcome. In a separate analysis we found an increased number of recurrences by cystoscopy if information of a positive urine test was communicated to the urologist, but not if the result was kept blinded. Hence the increased detection rate in the unblinded study arm ('intervention arm') could not be attributed to a high accuracy of the urine test per se, but to a diagnostic review bias (**chapter 7**). We concluded that communication of a positive urine test outcome to the urologist is associated with a substantial diagnostic review bias. This will lead to an underestimation of the sensitivity of a urine test when the urologist is blinded for a positive test outcome while performing cystoscopy. Indeed, in our CEFUB trial the sensitivity of the MA test increased from 29% when the urologist was blinded to 70% if unblinded for the MA outcome. As a consequence, the interpretation of the results of any (newly developed) point of care urine tests should take this potentially strong bias into account. Obviously, the increased detection of recurrences in the intervention arm as a consequence of awareness of the positive MA outcome at the time of cystoscopy undermines the notion that cystoscopy represents the "gold standard" for surveillance. It clearly shows that the sensitivity of cystoscopy is operator dependent and recurrences can be missed. Indeed, some studies comparing traditional white light cystoscopy with photodynamic blue light cystoscopy also suggested that the latter method is more sensitive not only for detection of flat bladder lesions, but also for papillary lesions[22]. To our opinion, these studies even underestimated the lack of sensitivity of white light cystoscopy, since their results are based on the comparison of the two procedures performed consecutively and it is clear from the above study that this may again lead to diagnostic review bias.

Upper Urinary Tract Tumours

Another objective of the randomized trial was to investigate whether the surveillance by MA would increase the number of detected upper urinary tract recurrences. Previous studies pointed at this possibility. In the intervention (MA) arm of our trial the number of imaging procedures to detect an upper tract UC was larger than in the conventional cystoscopy arm. This was a consequence of the protocol requiring a diagnostic work-up of the upper urinary tract if two consecutive MA tests were positive while cystoscopy was negative. The increased number of upper urinary tract cancers detected in the intervention arm was in line with our assumptions.

How does surveillance affect patients with NMI UC

The preference of about 90% of patients to opt for cystoscopy, if an alternative test would not offer a higher sensitivity than 90%[23] can be explained by the relatively low (1.30 on a scale from 1-3) overall patient perceived burden of cystoscopy and the absence of physical symptoms (fever, haematuria, urinary tract infection) as measured in the CEFUB trial (**chapter 8**). These observations are in contrast to the usually held views on the impact of cystoscopy on the patients' quality of life. Most papers advocating the substitution of cystoscopy by a urine test refer to the invasive nature of the procedure, causing much discomfort and to the higher risk of urinary tract infections.

The patients with a NMI UC reported a larger proportion (54%) of sexual dysfunction as ever been described. The cross-sectional analysis in **chapter 9** showed the prevalence of sexual function in patients just diagnosed with NMI UC before the start of the intensive surveillance scheme. Whether the sexual function is impaired by the diagnosis or by the impact of surveillance remained unanswered and must be assessed in future longitudinal studies. Besides, we report the unique finding that a considerable amount of patients is mistakenly afraid to harm their partner by sexual contact; the questionnaire series used in chapter 9 however does not elaborate on the fear to inflict harm to a partner caused by being a bladder cancer patient and with the prospect of intensive surveillance by cystoscopy. This latter question is very important and must be answered by extended questionnaires filled out by patients and their partners in a longitudinal prospective setting (M. Bekker).

The Balance of Costs and Effects

The previously mentioned view that it is not feasible to implement the MA test in the routine practice of surveillance of patients with NMI UC is further supported by our economic analysis. The latter clearly shows that the inclusion of the MA test leads to a substantial increase in expenses (about € 1000.- per capita over a 2-year follow-up period), while the total costs of a single cystoscopy in 2007 is € 167.-. It must be considered that even if by automatization and other reductions in expenses for the MA urine test the costs of the urine test would be less than cystoscopy this would not solve the issue of low sensitivity the test and patient preference for a more reliable test. The Medical Services Advisory Committee of the Australian government (link: http://www.tripdatabase.com/) issued a report in 2006 regarding the cost-effectiveness of the UroVysion FISH test and they concluded that at 5 years, the cost of implementation of the UroVysion pathway was Australian $7835, compared to $5959 for the conventional practice, while improved health outcome was not shown. Therefore, implementation of the

UroVysion FISH test increased the expected cost for patients until first recurrence by $1876 over current practice.

Future Perspective

1. Risk stratification

The inclusion of a number of clinico-pathologic variables in an easy tool for clinical decision taking helps to stratify patients for an individually tailored approach (Sylvester; www.eortc.be). Using the data of our prospective trial we anticipate the generation of a nomogram including WHO 2004 and FGFR3 mutation status, which could serve as a prognosticator for progression. Gene expression profiling studies may help to find additional prognosticators for progression. A main drawback is that low-grade UC have a very low progression rate, i.e. about 5-10% in 5 years. Thus, data of large numbers of patients are required to identify useful biomarkers. The possibility to progress in disease is a phenomenon with a static behaviour, which may be captured early in the genomic blueprint of a tumour and can consequently be identified early by tumour and patient characteristics. In the low-grade UC tumour group recurrent disease is seemingly unrelated to progressive disease. Not because early interception has prevented the disease to progress but because progressive disease is not in their genomic possibilities; as is recurrent disease an epiphenomenon.

For the prediction of recurrence rate no current marker identified in the tumour sample satisfies the requirements. Recurring disease can be considered a dynamic process, which signs can be intercepted by a urinary test in the course of the development of the tumour. We showed that a positive result of the urinary MA test often antedated, as also proven in studies with other urinary tests, a cystoscopically visible tumour lesion as we illustrated in figure 6.3 in chapter 6. This in contrast to other bladder tumours that were instantly visible at the time of the first positive MA-test result. Moreover, in spite of our limited data, these characteristics seemed to be patient dependent (data not shown). Maybe some neoplastic proliferations remain for some period of time in a subclinical, flat and non-papillary, stage before becoming visible for cystoscopic examination. This theory can be supported by the genetic evolutionary trees (van Tilborg, Kompier), which show that the primary tumour not always represents the first tumour. Future research must focus on the identification of the biological and molecular features that determine the velocity of a subclinical, latent urothelial lesion to its visibility.

Maybe most biomarker studies have even focused on the wrong cells (that is the tumour) to find useful tissue biomarkers for prediction of recurrence and instead more focus should be on the "morphologically normal" urothelium. The finding of an expression gene signature in the normal bladder mucosa in patients consistent with the signature specific for carcinoma in situ in patients with high-grade disease may also point into this direction[8].

2. Future Developments in Genetic Tests and Therapies

Of course future research should be focused on the development of more sensitive and specific markers than the current ones. A better selection of the polymorphic markers currently in use may be of help, but also inclusion of the FGFR3 mutation assays as well as the potential of proteomics and methylated DNA assays must be considered. Since recurring disease is a dynamic process and our urinary MA test proved to be able to predict recurrent behaviour, may be genetic urine tests can help us to identify those patients that need more intensive surveillance by cystoscopy and as such they may stratify them in low and high risk of recurrence. As to date qualifying urinary markers would be the MA urinary test, UroVysion FISH and the promising FGFR3 urinary test.

To compare two different urinary tests, for example MA and FGFR3 urinary test, a prospective randomized clinical trial could be performed in which five patient groups with primary or recurrent NMI UC (PUNLMP, pTa G1/G2 and pTa/pT1 LG tumours; stratified for FGFR3 gene status of tumour, stage, grade '73 and '04 and hospital) are monitored by five different surveillance schemes in which cystoscopy is in any case performed at 3, 12 and 24 months after inclusion; 1) FGFR3 urinary test followed by cystoscopy in case of positive test result, 2) MA urinary test followed by cystoscopy in case of positive test result, 3) FGFR3 combined with MA urinary test followed by cystoscopy in case of any positive test result, 4) FGFR3 combined with MA urinary test followed by cystoscopy regardless of urinary test result and 5) performing 3-monthly FGFR3 urinary test and MA test blinded for urologists next to 3-monthly cystoscopy scheme. FGFR3gene status of the recurrent tumour must be determined and patients remain in their randomized group after proven recurrent disease.

From a technical point of view much more effort should be put into effect to the increase in the effectiveness of the assays, like automatization and high throughput approaches in order to reduce the costs. It is also mandatory to reduce substantially the proportion of urine samples inadequate for (DNA) analysis. This could be achieved by improving both the logistics and the preservation methodology of the urine samples. Based on the results of our study we surmise, however, that the introduction of a urine test in current clinical setting replacing a substantial proportion of the cystoscopies is a long-term goal.

We might consider, however, for the short term future that we should not really focus on the reduction of the number of cystoscopies, but rather on the prevention of recurrences by a better eradication of abnormal cells and a more effective and more focused therapy. Like we showed in our study, others have also demonstrated that a TUR of the bladder contributes most to the costs of surveillance (and probably also to patient burden). Therefore it might be better to focus our research efforts more to treatment of the disease, may be already before a visible tumour is present. A German study recently showed the potential of photodynamic

transurethral resections to detect more and earlier lesions. The radical removal of early lesions reduced the number of recurrences and therefore proved to be cost-effective[24]. Knowles has generated optimism that therapies targeting the receptor tyrosine kinase of FGFR3 may have major application in the treatment of UC[25]. With improvements in specificity, or the use of sequential tests, such approaches may provide a cost-effective means of 'screening' for bladder cancer in a high-risk population.

Concluding Remarks

Based on the findings of these studies, it is my opinion that emphasis should be given to research on the development of a combination of tools (e.g. nomograms) to allow a tailor made surveillance scheme for individual patients with non-muscle invasive low-grade bladder cancer. This includes the determination of the mutation status of the fibroblast growth factor 3 genes during the pathological work-up of the tumour, the pathological determination of the differentiation grade by both the WHO '73 and '04 and substaging of the superficially invasive UC (pT1). The cystoscopic examination will remain an important 'confirmer' but must be challenged by technical improvements and new methods (like the promising fluorescence labelled cystoscopy). Studies have shown that improved bladder cancer detection during surveillance may reduce long-term recurrence rates and costs of treatment[26]. Bladder tumour markers and cystoscopic examination are both valuable in surveillance, they do not perform a neck-and-neck race but wage a fight for implementation in an optimal surveillance scheme in routine clinical setting.

Hence, in the near future we should focus predominantly on the identification of NMI UC with a significantly higher risk for clinical progression, secondly develop a decision model based on these risks for clinical progression and offer these patients an individualized scheme of surveillance with a combination of cystoscopic examinations (optimized) and urinary tests (tumour and patient specific), finally the focus must lie on the detection and treatment of clinical relevant tumours.

References

1. Botteman MF, Pashos CL, Redaelli A, Laskin B, Hauser R. The health economics of bladder cancer: a comprehensive review of the published literature. Pharmacoeconomics 2003;21:1315-30.
2. Schrag D, Hsieh LJ, Rabbani F, Bach PB, Herr H, Begg CB. Adherence to surveillance among patients with superficial bladder cancer. J Natl Cancer Inst 2003;95:588-97.
3. Sylvester RJ, Van Der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, et al. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 2006;49:466-5; discussion 75-7.
4. Fitzpatrick JM, West AB, Butler MR, Lane V, O'Flynn JD. Superficial bladder tumors (stage pTa, grades 1 and 2): the importance of recurrence pattern following initial resection. J Urol 1986;135:920-2.
5. Lindgren D, Liedberg F, Andersson A, Chebil G, Gudjonsson S, Borg A, et al. Molecular characterization of early-stage bladder carcinomas by expression profiles, FGFR3 mutation status, and loss of 9q. Oncogene 2006;25:2685-96.
6. Holmang S, Andius P, Hedelin H, Wester K, Busch C, Johansson SL. Stage progression in Ta papillary urothelial tumors: relationship to grade, immunohistochemical expression of tumor markers, mitotic frequency and DNA ploidy. J Urol 2001;165:1124-8; discussion 28-30.
7. Hernandez S, Lopez-Knowles E, Lloreta J, Kogevinas M, Amoros A, Tardon A, et al. Prospective study of FGFR3 mutations as a prognostic factor in nonmuscle invasive urothelial bladder carcinomas. J Clin Oncol 2006;24:3664-71.
8. Dyrskjot L, Zieger K, Real FX, Malats N, Carrato A, Hurst C, et al. Gene expression signatures predict outcome in non-muscle-invasive bladder carcinoma: a multicenter validation study. Clin Cancer Res 2007;13:3545-51.
9. Barbisan F, Santinelli A, Mazzucchelli R, Lopez-Beltran A, Cheng L, Scarpelli M, et al. Strong immunohistochemical expression of fibroblast growth factor receptor 3, superficial staining pattern of cytokeratin 20, and low proliferative activity define those papillary urothelial neoplasms of low malignant potential that do not recur. Cancer 2008;112:636-44.
10. Lopez-Beltran A, Luque RJ, Alvarez-Kindelan J, Quintero A, Merlo F, Requena MJ, et al. Prognostic factors in survival of patients with stage Ta and T1 bladder urothelial tumors: the role of G1-S modulators (p53, p21Waf1, p27Kip1, cyclin D1, and cyclin D3), proliferation index, and clinicopathologic parameters. Am J Clin Pathol 2004;122:444-52.
11. Paner GP, Ro JY, Wojcik EM, Venkataraman G, Datta MW, Amin MB. Further characterization of the muscle layers and lamina propria of the urinary bladder by systematic histologic mapping: implications for pathologic staging of invasive urothelial carcinoma. Am J Surg Pathol 2007;31:1420-9.
12. Ooms EC, Anderson WA, Alons CL, Boon ME, Veldhuizen RW. Analysis of the performance of pathologists in the grading of bladder tumors. Hum Pathol 1983;14:140-3.
13. Kurth KH, Denis L, Bouffioux C, Sylvester R, Debruyne FM, Pavone-Macaluso M, et al. Factors affecting recurrence and progression in superficial bladder tumours. Eur J Cancer 1995;31A:1840-6.
14. Van Rhijn BW, Vis AN, Van Der Kwast Th, Kirkels WJ, Radvanyi F, Ooms EC, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. J Clin Oncol 2003;21:1912-21.
15. Van Rhijn BW, Van Der Poel HG, Van Der Kwast Th. Urine markers for bladder cancer surveillance: a systematic review. Eur Urol 2005;47:736-48.
16. Van Rhijn BW, Lurkin I, Chopin DK, Kirkels WJ, Thiery JP, Van Der Kwast Th, et al. Combined microsatellite and FGFR3 mutation analysis enables a highly sensitive detection of urothelial cell carcinoma in voided urine. Clin Cancer Res 2003;9:257-63.
17. Van Oers JM, Lurkin I, Van Exsel AJ, Nijsen Y, Van Rhijn BW, Van Der Aa MN, et al. A simple and fast method for the simultaneous detection of nine fibroblast growth factor receptor 3 mutations in bladder cancer and voided urine. Clin Cancer Res 2005;11:7743-8.
18. Amira N, Mourah S, Rozet F, Teillac P, Fiet J, Aubin P, et al. Non-invasive molecular detection of bladder cancer recurrence. Int J Cancer 2002;101:293-7.

19. Frigerio S, Padberg BC, Strebel RT, Lenggenhager DM, Messthaler A, Abdou MT, et al. Improved detection of bladder carcinoma cells in voided urine by standardized microsatellite analysis. Int J Cancer 2007;121:329-38.
20. Yoder BJ, Skacel M, Hedgepeth R, Babineau D, Ulchaker JC, Liou LS, et al. Reflex UroVysion testing of bladder cancer surveillance patients with equivocal or negative urine cytology: a prospective study with focus on the natural history of anticipatory positive findings. Am J Clin Pathol 2007;127:295-301.
21. Lopez-Beltran A, Alvarez-Kindelan J, Luque RJ, Blanca A, Quintero A, Montironi R, et al. Loss of heterozygosity at 9q32-33 (DBC1 locus) in primary non-invasive papillary urothelial neoplasm of low malignant potential and low-grade urothelial carcinoma of the bladder and their associated normal urothelium. J Pathol 2008;215:263-72.
22. Witjes JA, Douglass J. The role of hexaminolevulinate fluorescence cystoscopy in bladder cancer. Nat Clin Pract Urol 2007;4:542-9.
23. Vriesema JL, Poucki MH, Kiemeney LA, Witjes JA. Patient opinion of urinary tests versus flexible urethrocystoscopy in follow-up examination for superficial bladder cancer: a utility analysis. Urology 2000;56:793-7.
24. Burger M, Zaak D, Stief CG, Filbeck T, Wieland WF, Roessler W, et al. Photodynamic diagnostics and noninvasive bladder cancer: is it cost-effective in long-term application? A Germany-based cost analysis. Eur Urol 2007;52:142-7.
25. Knowles MA. Role of FGFR3 in urothelial cell carcinoma: biomarker and potential therapeutic target. World J Urol 2007;25:581-93.
26. Grossman HB, Gomella L, Fradet Y, Morales A, Presti J, Ritenour C, et al. A phase III, multicenter comparison of hexaminolevulinate fluorescence cystoscopy and white light cystoscopy for the detection of superficial papillary lesions in patients with bladder cancer. J Urol 2007;178:62-7.

CHAPTER 12

Summary and Samenvatting

The Dutch summary is partly adapted from

Toepassing van moleculaire diagnostiek bij de follow-up van patiënten met oppervlakkig blaascarcinoom, een onderzoek naar de kosten-effectiviteit. Gepubliceerd in Kanker, Tijdschrift Kanker Oktober 2002, p12-14.

Madelon N.M. van der Aa
Theo H. van der Kwast
Ellen C. Zwarthoff

Blaaskanker: een terugkerend probleem Gepubliceerd in Kanker, Tijdschrift Kanker Juni 2008, p16-19.

Ellen C. Zwarthoff
Madelon N.M. van der Aa
Tahlita C.M. Zuiverloon
Lucie Kompier
Ewout W. Steyerberg
Wim J. Kirkels
Chris Bangma
Theo H. van der Kwast

Summary

Urothelial cancer (UC) of the bladder is the fifth most common malignancy in the western world. With approximately 357,000 new cases worldwide, tumours of the urinary system provide a considerable contribution to the overall human cancer burden. At the time of first diagnosis approximately 80% of the patients presents with non-invasive papillary UC (pTa) or with superficially invasive UC (pT1). Combined the pTa and pT1 bladder cancers represent the non-muscle invasive urothelial carcinomas (NMI UC). Patients with newly diagnosed NMI UC will generally undergo local bladder preserving therapy like transurethral resection (TUR) and intravesical chemo/immunotherapy. The likelihood that these tumours recur is 70% in 5 years with its peak incidence in the first year after initial treatment. Up to about 30% of these recurrent tumours will present with higher histologic grade and about 5-10% will progress to muscle infiltrating (pT2) UC. Therefore patients are recommended to adhere to regular (urethra)-cystoscopic surveillance (UCS) to detect recurrent tumour at early age. (Urethro)-cystoscopy is an endoscopic procedure. Given the prevalence of NMI UC, this disease is costly to manage, particularly since both cystoscopy and particularly TUR need to be performed repeatedly. Two strategies can be envisioned that may allow the reduction of the cystoscopy frequency during bladder cancer surveillance: 1) the reliable identification of a subset of patients with NMI UC with a low frequency of recurrence and progression using clinic-pathological disease variables (described in **Part II**), and 2) establishment of novel test systems on voided urine samples with a sensitivity and specificity for the detection of bladder cancer recurrences that can match cystoscopy (see **Part III**). It is commonly held that these developments are propelled by 1) the burden of cystoscopy as perceived by patients (see **Part IV**) and 2) the potentially lower costs of new techniques as compared to current follow-up schemes using cystoscopy (topic of **Part V**).

Knowledge of the biologic behaviour of newly diagnosed bladder cancer might enable the identification of subgroups of patients to offer a tailor-made surveillance scheme. Part II of the thesis describes the prognostic impact of conventional clinic-pathologic markers and a few selected novel biomarkers for NMI UC. For decades conventional parameters, being the clinical and histological features of the primary tumour, are evaluated in order to provide prognostic information to the patient. **Chapter 3** covers a meta-analysis of several large studies quantifying the predictive value of these conventional factors. This meta-analysis demonstrates that none of the conventional clinic-pathologic parameters are good prognosticators for recurrence of bladder cancer, but that both progression and mortality were strongly affected by the histopathological tumour grade, as defined by the WHO 1973 bladder cancer grading system, and by the presence of carcinoma in situ. One of the most promising novel prognosticators of NMI UC is the mutation status of the Fibroblast Growth Factor Receptor 3 (FGFR3) gene. In UC the presence of this mutation is strongly associated with genetically

stable bladder cancer and with low-grade, low stage disease. **Chapter 4** analyses the prognostic value of both the grading systems according to the classification of WHO 1973 (G1, G2, G3) and 2004 (PUNLMP, low-grade, high-grade) as well as biomarkers FGFR3 mutation status, CK20 and Ki-67 in a prospectively collected series of 221 patients with low stage (pTa, pT1) bladder cancers. None of the parameters could predict disease recurrence, but the two grading systems both contributed valuable independent information for disease progression. For this reason, pathologists might consider to report the tumour grade according to both grading systems. FGFR3 mutation status was able to discriminate progressors from non-progressors in a subset of patients with high-grade UC. The combination of WHO 2004 grading combined with FGFR3 status allows a better risk stratification for patients with pTa/pT1 and high-grade NMI UC. For this reason we suggest that it may be of clinical value to implement on a routine basis the FGFR3 mutation status in pathological reporting of NMI UC.

Patients with superficially invasive (pT1) papillary UC may run a highly variable disease course, creating a challenge for urologists on the choice of optimal treatment of patients with this bladder cancer. Several attempts have been made for substaging of UC in order to identify the clinical aggressive tumours, but they suffer of poor reproducibility. For this reason the current WHO 2004 classification system of bladder tumours does not include substaging of pT1 UC. We present a new substaging system in **chapter 5,** based on an easy assessment of the extent of invasion, distinguishing micro-invasive and extensive invasion of the subepithelial stroma. Unifocal or micro-invasive (pT1mic) is defined by a single focus of carcinoma, invading over a maximum distance of 0.5mm, and extensive pattern of invasion (pT1ext) represents either more than one focus of invasion and/or extensive infiltration of the subepithelial stroma over 0.5mm). The presence of pT1ext at initial diagnosis proved to be the strongest predictor for progression. We tested the diagnostic reproducibility and found 81% agreement. Mutant FGFR3 was commonly observed in pT1mic UC (63%) but rarely in pT1ext UC (7%), underlining the biological relevance of this simple substaging system. Further studies are warranted to validate this substaging system in a separate series of pT1 bladder cancers.

Worldwide many research groups continue to search for diagnostic tests on voided urine samples (urine tests) allowing the detection of bladder cancer recurrences during the surveillance of patients with NMI UC. These tests should be at least as accurate, robust and cost-effective as cystoscopy, the current gold standard for the detection of recurrent bladder cancer in patients with NMI UC. This thesis addresses the important issue of the feasibility and cost-effectiveness of surveillance of patients with NMI UC. **Part III, IV** and **V** report the outcomes of a randomized multicenter prospective trial on the feasibility and cost-effectiveness of an alternative surveillance strategy in the follow-up of patients with low-grade (WHO 1973 grade 1 and 2) NMI UC (CEFUB trial) conducted in the region of Rotterdam.

The CEFUB trial evaluated two surveillance schemes; surveillance by a conventional regimen (control arm) and by the follow-up of patients in whom the (conventional white light)

cystoscopies at 6, 9, 15, 18 and 21 months of follow-up after the last recurrence were replaced by a genetic urine test, i.e. the microsatellite analysis (MA) test, on DNA extracted from voided urine samples (test or intervention arm). Primary outcome measure was the number of tumour recurrences in the patients randomized to the test arm and the control arm. If significantly more tumour recurrences were found in the test arm this might imply that the MA test is more sensitive than cystoscopy. Secondary outcome measures were 1) the quality of life during follow-up of the patients, including physical complaints as a consequence of the diagnostic intervention, 2) comparison of the cost-effectiveness of the two arms of the trial and 3) the detection of upper urinary tract carcinomas.

Patients with primary or recurrent low-grade NMI UC (i.e. TNM 1997 stage pTa, pT1, and WHO 1973 grade 1 or 2) were recruited by 10 Dutch hospitals for participation in the randomized CEFUB trial (Cost-Effectiveness of Follow-up of Bladder Cancer Trial). All participating urologists were informed about the literature on the performance of the MA test for the detection of recurrent UC. Patients with a history of carcinoma in situ or WHO 1973 grade 3 UC were excluded from participation. After transurethral resection (TUR) of a primary or recurrent bladder cancer, 448 patients were randomized after signing an informed consent form (registration: ClinicalTrials.gov NCT00126958). The patients were stratified for hospital (ten hospitals), histopathological diagnosis (grade and stage) and *FGFR3* gene mutation status (mutation, wild type). Participants were assigned to two trial arms by block randomization in order of appearance. Urologists of attendance received Case Record Forms with a unique trial number linked to a database in the center of coordination. Study started at the first cystoscopy at 3 months after the TUR of inclusion with a follow-up either by 3-monthly cystoscopy alone (control arm) or by cystoscopy at 3 months, 12 months and 24 months and 3-monthly urine tests on voided urine samples (intervention arm). The urine test on voided urine samples, i.e. microsatellite analysis (MA), was performed on patients of both the control and intervention arm. The laboratory MA tests were done in a central laboratory (Department of Pathology, Erasmus MC, Rotterdam) without knowledge of clinical or histological status. Urine test results were only communicated (by mail) to the urologist of attendance in the test arm, and according to protocol a positive test was followed by a cystoscopy within a few weeks. If positive urine test outcome was incongruent with cystoscopy during two subsequent follow-up visits the protocol recommended imaging of the upper urinary tract as this raised the possibility of UC in the ureter or in the pelvicalyceal part. The primary outcome of the study was tumour recurrence, defined as the presence of a histopathologically confirmed UC detected by a cystoscopy during the follow-up.

Part III comprises **chapter 6** and **7** and describes the results of the randomized trial (CEFUB). In **chapter 6** we describe the feasibility and clinical utility of Microsatellite Analysis (MA) on voided urine samples in the setting of the CEFUB trial, comprising 14 outpatient clinics (in 10 hospitals), for the detection or prediction of NMI UC bladder cancer recurrences. Although the

vast majority of urine samples (81%) were of sufficient quality to allow MA testing, transportation times longer than 48 hours to the central laboratory adversely affected the proportion of samples giving satisfactorily results. Thus, adequate logistics are required prior to implementation of MA testing in a routine setting. Sensitivity and specificity of the MA urine test for detection of a recurrence at the time of the subsequent cystoscopy within a few weeks after the urine test (cross sectional analysis) was 58% and 73%, respectively. The positive predictive value of MA was only 20% in cross-sectional analysis. However in longitudinal analysis, the 2-year risk to develop a recurrence reached 83% if in a patient the MA outcome at consecutive urine tests was persistently positive and 22% when persistently negative. Moreover we found a higher detection rate for recurrences of the MA-test in male-smoking patients, that is a high number of false negative moments in females and non-smokers. Tumours of the pyelo-caliceal system and ureter were also preceded by a positive MA test. We concluded that consecutive positive MA results are a strong predictor for future recurrences. However, the main finding of our study is the relatively low cross-sectional sensitivity and specificity, so MA testing on voided urine samples is not sufficiently accurate to recommend its implementation in routine clinical practice. In spite of the limited accuracy of the MA test for detection of bladder cancer recurrences, the number of recurrences detected in the intervention arm exceeded that of the control arm (primary outcome parameter of the CEFUB-trial). During a median follow-up time of 34 months, 257 recurrences were detected in the intervention arm, compared to 205 in the control arm (log rank p=.008). We suggest that this can be explained by the unblinding of the urologist for the MA outcome in the intervention arm, while in the control arm the attending urologist was not informed about the outcome of the urine test. We considered in **chapter 7** the possibility that the awareness of a positive MA outcome may have prompted a higher detection rate of UC recurrences by cystoscopy. This phenomenon is also known as diagnostic review bias. Indeed, separate analysis of the patients of the intervention arm showed a much higher cross-sectional sensitivity (70%) of the MA test compared to that of the control arm (29%). Further, there was no difference in detection of recurrences between the two trial arms when urine test results were negative. We conclude that communication of a positive urine test outcome to the urologist is associated with a substantial diagnostic review bias. Our findings imply that cystoscopy may not be as reliable as generally thought, detracting from its role as the gold standard. As a consequence, the interpretation of the results of (newly developed, possibly point-of-care) urine tests should take this potentially strong bias into account.

Part IV focuses on the perspective of the bladder cancer patients by evaluating the outcome of questionnaire studies addressing the effects of surveillance by cystoscopy and urine testing on the perception of their quality of life. The study was a part of the randomized CEFUB trial. **Chapter 8** contains the analysis of the burden of cystoscopy as perceived by patients under regular surveillance for NMI UC during the different stages of the cystoscopic examination and in the subsequent week. The overall burden of cystoscopy was mild, i.e. a score of 1.3

on a scale from 1-3, mainly as the consequence of pain and discomfort, particularly during the introduction of the scope. The burden of MA appeared fully attributable to the waiting time for the test result. Not surprisingly, the average overall burden was significantly higher after cystoscopy than after a urine test as surveillance test. Notably, physical symptoms like urinary tract infections, fever and haematuria were very rare in our NMI UC patient group, in contrast to previous findings. Therefore, it is not surprising that patients will adhere to cystoscopic surveillance as long as cystoscopy is considered the gold standard. In **chapter 9** we describe the prevalence of sexual dysfunction in our patients under surveillance with NMI UC and evaluated the risk factors. Patients were asked to fill in questionnaires, four weeks after diagnosis. The prevalence of sexual dysfunction in patients with NMI UC is high (54%) compared to an age and gender matched healthy population (20-45%). Diagnosis of NMI UC can be considered as a diagnosis of a chronic disease; therefore long-term sexual performance of these patients and partners would be likely to benefit from proper sexual information in the outpatient clinic. However, whether the sexual function is impaired by the diagnosis or by the impact of surveillance remained unanswered.

Part V concerns the societal perspective of the effects of alternative surveillance schemes in patients with bladder cancer as studied in during our randomized CEFUB trial. In **chapter 10** we determined how to achieve cost-effectiveness in the surveillance of NMI UC by replacing a proportion of cystoscopies for MA testing on voided urine samples during surveillance of patients under surveillance for NMI UC. The costs and effects (probability of being in a specific health state) were compared for the two trial arms. Various sensitivity analyses were performed to determine the MA sensitivity, MA specificity, and costs of MA urine for which the test arm was as cost-effective as the conventional arm. The test arm led to higher costs (€4,104 versus €3,433 per capita). The test arm would be as effective as the conventional arm if the sensitivity of the currently available MA-markers increased at least to 63% and would cost the same if the costs of MA-urine decreased from €158 to at most €130.50.

It is our opinion that the research on the development of a combination of tools (e.g. nomograms) should be emphasized to achieve a better risk stratification and to allow a tailor made surveillance scheme for individual patients with NMI low-grade UC. The determination of the mutation status of the FGFR3 gene in the tumour during the pathological work-up of the tumour may become routine and its outcome may be included such a nomogram. Urine testing improvements should both become focused on a better selection of molecular markers, future urine tests may also include the detection of FGFR3 mutant DNA and much emphasis needs to be put in the reduction of the costs of an alternative urine test. Although the cystoscopic examination will remain important but technical improvements and new visualization methods may allow a better detection of recurrences.

Samenvatting

Kanker van de urinewegen wordt per jaar bij ongeveer 4550 mensen in Nederland geconstateerd. De urineblaas, de plasbuis, urineleiders en het nierbekken zijn bekleed met urotheel en tumoren kunnen op al deze plaatsen ontstaan. Verreweg de meeste urotheelceltumoren worden echter in de blaas gevonden (90%). Blaascarcinoom is een vorm van kanker die 4 keer vaker bij mannen dan bij vrouwen voorkomt. De belangrijkste risicofactor voor blaaskanker is roken. Het risico om blaaskanker te krijgen is bij rokers 2-6x groter dan bij niet rokers. Waarschijnlijk is dit de reden dat meer mannen dan vrouwen blaaskanker krijgen. De tumoren worden meestal ontdekt doordat de patiënt bemerkt dat hij/zij bloed bij de urine heeft en hiermee naar de huisarts gaat. De huisarts zal in het algemeen eerst onderzoeken of de bloeding door een ontsteking komt. Is dit niet het geval, dan wordt de patiënt doorverwezen naar een uroloog. Deze onderzoekt de blaas met een cystoscoop (camera) die via de plasbuis wordt ingebracht.

In 95% van de blaastumoren betreft het een carcinoom (kanker) van de urotheelcellen. Het urotheelcelcarcinoom vertegenwoordigt een breed spectrum aan pathologische diagnoses, van oppervlakkig groeiend en goed-gedifferentieerd (Ta G1) tot carcinoma in situ (CIS) en spierinvasief, slecht-gedifferentieerd (T2-4 G3). Blaastumoren worden ingedeeld in stadia afhankelijk van de mate waarin ze doorgegroeid zijn in het urotheel of de andere weefsellagen van de blaas. Een Ta tumor is een poliepachtige (wratachtige) tumor die zijn basis heeft in de urotheellaag. Carcinoma in situ (CIS) is een vlakke laesie die alleen in de urotheellaag groeit. In de stadia T1-T4 zijn de tumoren verder doorgegroeid in de blaaswand. Ta en CIS worden de niet invasieve tumoren genoemd. Ta, T1 en carcinoma in situ (CIS) worden samen de niet-*spier*invasieve tumoren genoemd. Stadia T2-T4 zijn spier-invasief en het is mogelijk dat er dan uitzaaiingen zijn in lymfeklieren of elders. Als de tumor zich nog in de blaas bevindt zal behandeling van spierinvasieve tumoren bestaan uit het weghalen van de hele blaas (cystectomie), radicaal met aanliggende organen (prostaat en uterus) en met omringende lymfeklieren. De 5 jaars overleving voor patiënten met T2 tumoren die behandeld zijn met een radicale cystectomie is 45%, dit is lager voor de stadia T3 en T4.

De niet-spierinvasieve tumoren vormen de meerderheid van de voor het eerst gediagnostiseerde blaaskankers (80%). De behandeling van deze vorm van blaaskanker bestaat uit het weghalen van de tumor door middel van een transurethrale resectie (TUR), dat wil zeggen dat via de plasbuis de tumor uit de blaas wordt weggesneden. De 5 jaars overleving van de oppervlakkige tumoren is zeer goed (90%). Een nadeel is echter dat ze vaak terugkomen (recidiveren). In 70% van de patiënten ontwikkelt zich opnieuw een tumor binnen 5 jaar en in 10% van de gevallen kunnen deze uitgroeien tot een spierinvasieve tumor. De recidieven ontwikkelen zich overal in de blaas, zelfs in de hogere urinewegen en in veel patiënten worden

er uiteindelijk meerdere recidieftumoren gevonden. Vanwege het hoge risico op recidivering en de kans op progressie tot een levensbedreigend spierinvasieve blaaskanker bij patiënten met de diagnose niet-spierinvasief urotheelcelcarcinoom (pTa/pT1) is een frequente follow-up noodzakelijk. Tot op heden geldt de cystoscopie als gouden standaard voor het opsporen van recidieven. In het eerste jaar na diagnose zullen patiënten 3-maandelijks worden gecontroleerd, na uitblijven van recidief kan de frequentie van controle eventueel afnemen naar 4-maandelijks, halfjaarlijks of zelfs jaarlijks. Nieuwe methoden waarbij met fluorescerende stoffen wordt gewerkt in combinatie met een andere belichting hebben aangetoond dat bij deze techniek meer vlakke blaastumoren, zoals met name het carcinoma in situ worden ontdekt. Vanzelfsprekend is cystoscopie niet in staat om recidieven in de hogere urinewegen (urineleiders, nierbekken) te detecteren.

Er worden met regelmaat studies gedaan om te zoeken naar andere mogelijkheden van follow-up bij patiënten met oppervlakkig blaascarcinoom. Belangrijke redenen om naar alternatieve methoden voor cystoscopie te zoeken zijn 1) de aanname dat patiënten het onderzoek ook daadwerkelijk als zeer belastend ervaren én 2) de verwachting dat alternatieve (urine)testen goedkoper zullen zijn dan de cystoscopie. Deze alternatieve methodes dienen echter even sensitief en specifiek te zijn als cystoscopie. Er zijn twee manieren om het huidige intensieve follow-up schema van scopiën minder belastend voor de patient te maken:1) door patiënten groepen te ontdekken die een minder frequente follow-up nodig hebben omdat de tumor als minder agressief kan worden gekarakteriseerd of 2) door een nieuwe test te vinden die afwisselend met de cystoscopy een minder intensief en belastend follow-up schema kan bezorgen. Zulke nieuwe urinetesten maken gebruik van de spontane urine van de patient, waarin zich tumor cellen of specifieke moleculen bevinden, die duiden op de aanwezigheid van een blaaskanker recidief.

In **deel II** van dit proefschrift worden karakteristieken van patiënten en tumoren aangetoond die gekoppeld zijn aan een gunstig of minder gunstig verloop van het tumorproces. In **hoofdstuk 3** worden alle vanouds bekende klinische en pathologische factoren met een voorspellende waarde (predictor) die tussen 1983-2004 in grote prospectieve studies zijn beschreven geanalyseerd. Uit deze meta-analyse blijkt dat het niet mogelijk is om met deze factoren de tijd tot het volgende recidief te voorspellen, maar de kans op progressie van de tumor kan hiermee wel redelijk voorspeld worden. In **hoofdstuk 4** worden naast de bekende pathologische voorspellers ook een aantal moleculaire markers onderzocht, zoals het optreden van een mutatie in het fibroblast groeifactor receptor 3 (FGFR3) gen.
Het FGFR3-gen behoort tot een van membraan gebonden celreceptoren, welke na binding aan fibroblast groei factor een signaal afgeeft aan de cel. Een mutatie van dit gen is de meest voorkomende kiemcelafwijking bij de mens, verantwoordelijk voor meer dan 7 erfelijke skeletafwijkingen, leidend tot dwerggroei. Het gen is gelokaliseerd op chromosoom 4 en wordt

door de mutatie geactiveerd. Ook in blaaskanker treedt frequent een activerende mutatie in het FGFR3-gen op. Mutaties in het FGFR3 gen worden gevonden in ongeveer de helft van alle urotheelceltumoren, met de hoogste frequentie in Ta tumoren (ongeveer 75%) vergeleken met de invasieve pT2-T4 tumoren (20%) of CIS (0%). Er zijn 11 verschillende mutaties gevonden in 3 delen van het gen. In ons laboratorium hebben we een test ontworpen die alle mutaties in een keer kan aantonen. Deze test kan zowel uitgevoerd worden op het DNA afkomstig van de in paraffine ingebedde tumor als op DNA geëxtraheerd uit urine. De sterke associatie tussen FGFR3-mutatie en laaggradige tumoren suggereert het bestaan van een subgroep van patiënten met beter verloop van de niet-spier invasieve blaastumoren. Dit zou kunnen betekenen dat deze groep patiënten in hun follow-up minder vaak een cystoscopie of zelfs TUR behoeven te ondergaan. In **hoofdstuk 4, 5** en **6** van ditproefschrift laten we zien dat de FGFR3 mutatie een prognostische gustige groep van blaaskankers identificeerd binnen de groep van hooggradige blaaskankers, zoals gekarkateriseerd door het meest recente graderingssysteem (WHO 2004). Ook lieten we zien dat het mogelijk is om het beloop van de klinisch problematische groep van pT1 blaaskankers beter te voorstellen door toepassing van een nieuw systeem van substageren.

Het is al langer bekend dat tumorcellen in urine te vinden zijn. In 1864 werden voor het eerst neoplastische urotheelcellen in urine herkend. Pas halverwege de vorige eeuw beschreven Papanicolaou en Marschall het gebruik van cytologie voor de diagnose van urotheelcelcarcinoom. Tumorcellen kunnen door de patholoog worden onderscheiden van normale cellen die zich eveneens in urine bevinden (cytologie). Sinds die tijd wordt cytologie in combinatie met cystoscopie in veel centra gebruikt als screeningsmethode voor blaascarcinoom. Cytologie is echter weinig gevoelig voor detectie van laaggradige niet-spierinvasieve blaascarcinomen (30-60% sensitiviteit). Aangezien de meeste blaaskanker recidieven laaggradig zullen zijn, heeft cytologie maar een beperkte waarde bij de surveillance van patienten met een laaggradige niet-spierinvasieve blaaskanker. Het zijn de hooggradige, de meer invasieve carcinomen en carcinoma in situ, die wel door middel van cytologie gediagnosticeerd worden. De zoektocht naar het ontwikkelen van nauwkeurige, snelle en prognostische methoden voor follow-up en naar methoden voor vroege detectie van blaascarcinoom hebben geleid tot de ontdekking van verschillende veelbelovende blaastumormarkers.

Een aantal van deze potentieel diagnostische markers zijn gebaseerd op tumor-geassocieerde antigenen, zoals de Blaas Tumor Antigen-test en Immunocyt, op een verhoogde productie van vasculaire endotheliale groei factoren, of op veranderingen in celmorfologie, de cytoskelet markers. Ondanks grote vooruitgang in de ontwikkeling van deze markers voor detectie van blaastumoren zijn ze nog niet nauwkeurig genoeg om eventueel de cystoscopie te vervangen. Een nieuwe benadering die van het laatste decennium dateert is om tumorgebonden DNA veranderingen te gebruiken.

Het humaan genoom wordt gevormd door 23 chromosomenparen, waarbij elk paar bestaat uit een kopie van een maternaal en van een paternaal chromosoom. Het DNA in de chromosomen wordt gevormd door een opeenvolging van 4 verschillende basen (**A**denine, **T**hymine**, C**ytosine, en **G**uanine). De volgorde van deze basen codeert voor de belangrijke genetische informatie van een organisme. Een microsatelliet is een herhaling van een simpele base-volgorde van 2, 3 of 4 basen in het DNA. De totale lengte kan variëren tussen 15 en 20 herhalingen van diezelfde base-combinatie, bijvoorbeeld een (CA)n-repeat. Ze komen overal in het genoom voor, in allerlei lengtes en combinaties. Door de hoge mate van variatie tussen deze microsatellieten bestaan er van mens tot mens grote verschillen in het DNA. Voor kankeronderzoek is deze variatie van groot belang.

Microsatellieten zijn relatief instabiel en dit resulteert in verschillen in het aantal CA herhalingen waardoor de lengte van de microsatelliet varieert. Als de microsatelliet in lengte verschilt op de maternale en paternale kopieën van een chromosoom (heterozygotie) kunnen we dit lengteverschil aantonen. Is een van de twee chromosomen in de kankercel verloren gegaan, dan spreken we van verlies van heterozygotie, of LOH. Voor het urineonderzoek gebruiken we meerdere microsatelliet markers op verschillende chromosomen om de gevoeligheid te vergroten. LOH-analyse is een methode die gebruik maakt van de mogelijkheid om een microsatellietsequentie in het DNA te herkennen. Deze DNA veranderingen zijn met de huidige technologieën relatief gemakkelijk zichtbaar te maken. Bovendien zijn deze veranderingen aanwezig in de oppervlakkige blaastumoren, die niet met cytologie kunnen worden gedetecteerd doordat de cellen er normaal uitzien. In dit proefschrift wordt deze analyse getest en MA-test (Microsatellite Analyse Test) genoemd.

In een grootschalig onderzoek gefinancierd door ZONMW hebben we in samenwerking met 10 regionale ziekenhuizen de gevoeligheid van de MA test voor het opsporen van een blaaskanker recidief onderzocht op 815 urine monsters van patiënten die gevolgd werden in verband met een eerder verwijderde oppervlakkige blaastumor (**hoofdstuk 6**). In deze gerandomiseerde trial werden patienten met een laaggradige niet-spierinvasieve blaaskanker ingedeeld in twee groepen, te weten patienten die vervolgd werden op de gebruikelijke manier (cystoscopie) elke drie maanden na de diagnose (controle arm) en patienten bij wie een deel van de cystoscopieen werden vervangen door de MA test (test arm). Indien in de test arm evenveel recidieven zouden worden opgespoord als in de controle arm, bij gelijkblivende aantallen patienten met progressie zou de trial een voorkeur voor het alternatieve surveillance schema aangeven. Overigens werd in de controle arm ook een MA test uitgevoerd ten tijde van de cystoscopie, maar de uitslag daarvan werd niet meegedeeld aan de uroloog of patient.

We waren in staat om in de deelnemers aan de CEFUB trial bij wie urine test en corresponderende cystoscopie resultaten bekend waren 49 van de 84 recidieven aan te tonen (sensitiviteit 58%). Deze sensitiviteit was lager dan op basis van eerdere studies te verwachten

was. Uit deze cross-sectionele analyse (vergelijken van de diagnostische testen op hetzelfde tijdstip) bleek ook, dat er een groot aantal "vals positieven" was. Dat wil zeggen dat de MA test positief was en er op dat moment met cystoscopie geen tumoren werd gevonden. De positief voorspellende waarde (PPV) van de MA test was derhalve slechts 20%. Dit suggereert dat de test niet gevoelig is en vaak ten onrechte positief uitvalt. Hier kunnen we echter een aantal kanttekeningen bij plaatsen. De MA test bleek vaak eerder dan de cystoscopie recidief tumoren te kunnen detecteren. Vervolgden we de patiënten namelijk na een positieve MA test en een negatieve cystoscopie dan bleken ze enige tijd later toch een recidief tumor te hebben ontwikkeld. In de longitudinale analyse zien we dat de PPV van 20 naar meer dan 80% klimt als de urines positief blijven. De MA test heeft dus toch wel een voorspellende waarde en ook is ze in staat om blaaskanker in de hogere urinewegen op te sporen. Toch moeten we concluderen dat de test op dit moment niet goed genoeg is om aan te bevelen als een nieuwe routine test voor de surveillance van patienten met een laaggradige niet-spierinvasieve blaaskanker. Ondanks de lage PPV van de MA test bleek het aantal recidieven in de test arm van de trial duidelijk meer te zijn dan in de controle arm (cystoscopie follow-up alleen). Dit zou kunnen leiden tot de conclusie dat de urinetest toch een goed alternatief zou zijn. Echter, in de trial opzet was de uroloog in de test arm op de hoogte van de uitslag van de MA test voor het uitvoeren van de scopie, terwijl de uroloog van de patienten in de controle arm dit niet wist. Het is dus voorstelbaar dat de wetenschap van de uitslag van een positief urinetest van invloed kan zijn op de cystoscopische beoordeling van de blaas. In **hoofdstuk 7** wordt dit verder onderzocht en bevestigd. Voorkennis van de uitslag van een positive urinetest bleek een hogere detectie van recidieven op te leveren, en dit verschijnsel heet een diagnostische review bias. In het geval van een negatieve uitslag van de urine test was dit niet het geval. Dit leidt tot het onderschatten van de sensitiviteit van een urinetest als een uroloog is geblindeerd voor de resultaten. Deze bevinding is erg belangrijk in toekomstige onderzoeken van diagnostische urinetesten.

Verder vonden we dat de microsatelliet test gevoeliger was bij mannelijke patiënten en bij patiënten die geen FGFR3 mutatie in hun tumor hadden en/of rookten (**hoofdstuk 6**). Dit komt vermoedelijk doordat deze tumoren in het algemeen andere DNA afwijkingen hebben die juist met de MA test kunnen worden aangetoond. De meeste tumoren die werden gemist met de MA test waren kleine laaggradige Ta tumoren, maar ook 1 T1 graad 3 tumor. Daarentegen werden 4 tumoren van de urineleiders met de MA test gedetecteerd die niet zichtbaar zijn bij cystoscopie. De conclusies uit deze studies zijn dat meerdere positieve MA testen een sterke voorspeller zijn van een recidief blaastumor maar dat de gevoeligheid nog verbeterd kan worden bijvoorbeeld door selectie van patiënten en door combinatie met andere markers zoals FGFR3. Verder moesten we constateren dat een aantal markers niet goed werkte en dat we waarschijnlijk ook effectievere markers kunnen selecteren door bijvoorbeeld meer markers te kiezen uit gebieden van het genoom die in blaastumoren vaak verloren gaan. Dit zal in de nabije toekomst verder worden onderzocht. De MA test kan het niet goed doen als

er veel normale cellen in de urine zitten. Een beperking zal bovendien altijd zijn dat er niet in ieder urinemonster tumorcellen zitten. Een belangrijke bevinding is wel dat de MA-test tumoren in de hogere urinewegen kan aantonen die niet gezien worden met cystoscopie. Voor detectie van deze tumoren zijn andere, kostbare beeldvormende technieken zoals een CT scan nodig. Verder is het belangrijk dat de MA-test een voorspellende waarde heeft; een positieve test wordt vaak op enige termijn gevolgd door de ontdekking van een recidief.

Scopiëren is een endoscopische invasieve methode waarvan we denken dat het voor patiënten erg vervelend is. In **hoofdstuk 8** zien we dat een cystoscopie door de patiënt in geringe mate als onaangenaam wordt ervaren en er een kleine kans is op urineweginfecties en pijn bij het plassen in de dagen na de cystoscopie. Wel hebben blaaskanker patienten meer last van sexuele dysfunctie in vergelijking met andere patiënten populaties (**hoofdstuk 9**), maar het is niet duidelijk of dit veroorzaakt wordt door de cystoscopie of door de surveillance op zich.

In **part V** worden de effecten vergeleken met de kosten. Het kosten-vraagstuk is in de gezondheidszorg natuurlijk erg belangrijk. In **hoofdstuk 10** berekenen we daarom de kosten effectiviteit van de follow-up als de cystoscopien gedeeltelijk worden vervangen door de MA test. De controle arm is de groep patiënten die gecontroleerd wordt door middel van cystoscopie en in de test arm worden patiënten afwisselend gecontroleerd middels scopie en MA test (zoals eerder beschreven). De kosten en effecten (de kans om in een bepaalde gezondheids toestand te zijn) worden in beide armen met elkaar vergeleken. Vervolgens zijn er verschillende sensitiviteits analyses uitgevoerd om te bepalen bij welke sensitiviteit, specificiteit en prijs de beide armen aan elkaar gelijk zijn. De test arm was duurder dan de controle arm (€ 4104,= versus € 3433,= per capita). De test arm is berekend even effectief te zijn als de controle arm als de sensitiviteit minimaal 63% is en de kosten van een MA test zou dalen van € 158,= naar hoogstens € 130,50.

Naar aanleiding van het onderzoek beschreven in **part III** is duidelijk geworden dat er nog veel winst behaald kan worden uit de ontwikkeling van urine testen en de verbetering van de cystoscopie techniek. We moeten de nadruk leggen op de ontwikkeling van combinaties van methoden (nomogrammen) om tot een follow-up-op-maat te komen. De bepaling van de FGFR3 mutatie status van de tumor hoort bij de standaard work-up van de tumor door de patholoog. Uit preliminary results blijkt dat de combinatie van de FGFR3 en MA testen in staat is om in 75% van de gevallen een gelijktijdig recidief aan te tonen. De vraag is of deze sensitiviteit nog omhoog kan. De DNA testen zijn dus in staat om recidieftumoren eerder te ontdekken dan routine cystoscopie, maar de testen ontdekken niet alle tumoren en missen soms zelfs belangrijke tumoren. Ondanks het feit dat één derde van de patiënten de cystoscopie als een belastend onderzoek ervaart, kiest de meerderheid van de patiënten voor

de methode waarvan zij denken dat die de meeste zekerheid geeft. Wellicht is het de beste strategie om ons allereerst a) te richten op het herkennen van de groepen patiënten binnen de niet-spierinvasieve blaaskanker patiënten met een duidelijk hoger risico voor progressie en b) op basis van dit risicoprofiel een beslismodel te ontwikkelen waarbij gebruik wordt gemaakt van een follow-up schema waarin urinetesten en cystoscopiën (beide in geoptimaliseerde vorm) gecombineerd worden, waardoor c) de hoogste relevante detectiegraad wordt bereikt. Tenslotte duidt ons onderzoek op het belang van 1) het bepalen van de FGFR3 mutatie status en 2) het substageren van de groep van oppervlakkig invasief groeiende (pT1) blaaskankers een belangrijke rol kan spelen bij het bepalen van het risico op progressie.

CHAPTER 13

Acknowledgement / Dankwoord

Participants in CEFUB-trial

Contributing authors

List of Publications

Acknowledgement; een 'dankjewel' en bedankt

Dank u allen! Vooral u allen - patiënten - , bedankt allemaal en iedereen, voor de mogelijkheden, het geduld, de adviezen en de ondersteuning, de goede moed, de discussies, het plezier, de mooie momenten bij het koffiezetapparaat of in de koffiekamer en elk moment in Be-332, de heerlijke biertjes en de gezellige ontbijtjes, de bijzondere en minder bijzondere leermomenten, het geluk.

Bedankt!

- Madelon -

De schijnwerpers dansen door de zaal, een zaal vol mensen. Zo op het eerste gezicht lijken er meer mannen dan vrouwen aanwezig te zijn, en opvallend veel rokers eigenlijk. Vandaag mag dat blijkbaar. *Er wordt heel hard geapplaudiseerd!* Vanaf het balkon boven de ingang heb ik goed zicht op het feestelijke gebeuren. De zaal is rond, een warm koepelvormig licht plafond ademt lente-avond. Het is winter. In de ruimte rondom de vloer zijn, met een vrolijke diversiteit in hoogte en vorm, balkonnetjes gebouwd met sierlijke ballustrades. Een aantal wenteltrappen biedt toegang tot de verschillende balkonnen. Tegenover de ingang, aan de andere kant van de zaal, valt een prachtig mooie verhoging op met aan weerszijden een aantal treden om het podium te bereiken. Boven het podium hangt een gigantisch wit laken waarop in rood de letters 'C', 'E', 'F', 'U' en 'B' en moeilijker leesbaar iets van 'tiralalalaa'.

Er heerst een frivole, uitgelaten stemming. De schijnwerpers richten hun lichten op het podium waar even daarvoor is gestart met een officieel programma. Precies 484 mensen komen om de beurt op van links, ontvangen met een officieel gebaar en woord een glanzende CEFUB-sjerp en verlaten uitgelaten het podium van rechts onder luid geklap en gejoel. De wat schuchter ingezette polonaise zie je langzaam veranderen in een waar feestgedruis. Vol overgave wordt er gefloten, geklapt, 'Bravo' of 'Caramba' geroepen. *Het applaus voor de 484 mensen vanuit de zaal is oorverdovend!*

En dan klinken er ontkurkende geluiden. De schijnwerpers bewegen precies op het afgesproken moment gecoordineerd naar een balkon links van de ingang. De aanwezigen in de zaal zien nog net de verraste gezichten op het balkon nat worden van de bruisende champagne. Kurken vliegen door de lucht en confetti plakt aan warme wangen. Een jazz-combo speelt op het podium. De hele zaal proost, laat zich meeslepen door de muziek en kijkt naar het balkon waar de sjerpen uitgedeeld worden. Af en toe wordt er van herkenning, enthousiast, een naam uitgeroepen. Albert Schweitzer doet vol overtuiging de twist met aan weerszijde Jenneke Vis en Toine Zeegers. De enige echte Erasmus trekt, zoals altijd iets gebogen staande, zijn pij op en slaat onze Sint Franciscus zeer hartelijk op de schouder en roept iets uit over wij monikken en bruggen bouwen. De lof der efficiëntie, doortastendheid en schijnbare logistieke eenvoud gaan vervolgens uit naar Stefan Haensel en Egbert Boevé, boegbeelden van rust en klasse in de Haven. Clara en Amphia doen samen een moeilijke versie van de klompendans en maken daarbij zoveel mogelijk lawaai. Reinier de Graaff laat zijn follikels weer eens rammelen in een spetterend duet met dj maestro JFAT Bogdanovicz en als je goed kijkt zie je hier en daar enkele tranen van ontroering, prachtig. De zaal geniet en stemt volmondig in als er vervolgens een naamsverandering van Ikazia naar I-Kats-ia en van Vlietland naar Lie-land wordt voorgesteld. Verdiend! Het LUMC kijkt stilzwijgend toe en geniet terwijl Caroline de Jong-Mom haar prachtige rode schoentjes precies op de maat van het combo laat dansen. Een groot applaus zwelt op en als de jazzy klanken dan weer door de zwoele zaal klinken lijkt de schijnwerper te zoeken.

In het midden van dit hele spektakel tussen al die mensen en druppels deinen 3 opvallende gestalten, vrolijk lachend kijken ze geamuseerd om zich heen en dansen alle drie op geheel eigen wijze, erg grappig. Af en toe zwaaien ze, roepen ze wat in elkaars oor en lachen. Mensen komen op ze af en maken een praatje.

De eerste gestalte waar het licht opvalt, is een schijnbaar coordinatieloos bewegende lange tengere gestalte, die in één hand een fles Eiswein vasthoudt en met de andere drukgebarend een verhaal kracht bijzet. Als je goed luistert hoor je hem giechelen en de ene grap na de andere vertellen. Het is Theo, professor Theodorus H van der Kwast, patholoog in Toronto. "Thééééooooohooooo!" klinkt het ineens hard en ongepast door de zaal. Ik schrik want ik ben het zelf die zo roept! Oei, denk ik nog, wat nu! Ineens is het stil, zelfs het combootje zet onbedoeld een decrescendo in. Ik sta nog steeds op dat balkon te kijken, verschrikt nu, en zie hoe alle blikken zich langzaam vol verwachting op dat balkonnetje boven de ingang richten. En zie ook hoe de ingehuurde cabaretier verbaasd en zenuwachtig door zijn papieren bladert. Maar de ballustrade biedt geen enkele vorm van beschutting. "Héé Theeéoo," herhaal ik voorzichtig iets zachter, en nog zachter voeg ik daar "jij hier!?" aan toe. Theo is mijn held, mijn inspirator, mijn wetenschappelijk voorbeeld. Op Theo kun je bouwen en vertrouwen, Theo is bescheiden en helpt oprecht, Theo denkt verder en breder dan alleen zichzelf. Theo rent af en toe vol onschuld als een olifant door een porseleinkast, en dat vind ik ontzettend grappig! Theo werkt harder en denkt sneller dan het licht. Met Theo kun je paralelle gesprekken voeren, ongelofelijk lachen en heel veel grappen per minuut maken. Theo staat voor zijn liefde voor Veena en zijn zonen, Theo is verloren zonder Veena. Theo is Theo, uniek in zijn soort. Zonder Theo zou de CEFUB trial nooit zo succesvol zijn afgerond. Wat nu? Wat moet ik zeggen? Voordat de eerste 's-' tot SSSPEECH kan uitgroeien roep ik wanhopig met een tikje dramatiek: "Theo, jij was de drijvende kracht achter de CEFUB-trial, zonder jou was het geheel niet gebundeld geweest! Bedankt....(en iets harder en dramatischer) bédankt!!!" Het applaus zwelt weer aan en ik denk, professionaliteit, je moet professioneel blijven, dit is professioneel. Niet te emotioneel allemaal, straks gewoon even naar Theo toelopen en hem eens stevig bedanken.

Net naast de lichtbundel staat een man hard te klappen en te lachen. Af en toe lijkt hij wat te mompelen als "ja, dat is inderdaad zo, ja inderdaad...mmm..., als we de kansen op deze manier berekenen maakt dat een significant verschil, hoewel dat er misschien niet toe doet, precies, moet ik eens uitrekenen, ja leuk, moet een vrij simpel sommetje zijn eigenlijk.. mmmja....." Het is Ewout, professor Ewout W Steyerberg, klinisch besliskundige aan het Erasmus MC. Het licht van de schijnwerper draait nu zijn kant op en Ewout staat iets verlegen maar prachtig midden in de bundel. Het staat hem goed, licht, de lichtbundels lijken zijn gedachten te reflecteren. Nee niet weerkaatsen echt reflecteren. Golven van berekeningen en mogelijkheden worden teruggebeamd. Ewout heeft ook zoveel betekend voor de trial en voor mijn persoonlijke ontwikkeling, met herwonnen zelfvertrouwen roep ik zo hard als ik kan: "Applaus, applaus voor Eéééwout...!" Ontelbare besprekingen, eerst op de 20ste met

prachtig uitzicht later op de 2de met prachtig uitzicht. Altijd gezellig en altijd met iets lekkers, uren bomen, schematiseren, rekenen en discussieren om pas echt naar elkaar te luisteren als we allebei murw geluld waren. Het was een waar genoegen. Iedereen begrijpt wat er bedoelt wordt en stampende voeten, lucht-tong-en-tand-technieken worden aangewend om het heersende gevoel kracht bij te zetten. En het applaus is oorverdovend.

Weer lijkt de schijnwerper zijn licht te willen verplaatsen, naar iemand die vlak naast Ewout staat, het is een vrouw. De vrouw kijkt en observeert kritisch, lijkt het, met haar rechter wijsvinger draait zij zachtjes rondjes rondom haar lippen en met de andere hand houdt zij gedistingeerd een glas van de beste Bordeaux vast. Ze geniet van de aandacht, van de wijnen, het overheerlijke Franse en Italiaanse eten, ze geniet van de resultaten en gelooft krachtig in haar produkt. Haar produkt, de urine analyse, de 'simpele' methode om mutaties aan te tonen. Het is Ellen, Professor Ellen C Zwarthoff, biochemica. En ik roep aanzwellend "...én...Elluhuhuhn!..." Inmiddels staat ook Ellen in het volle licht en het applaus zet zich voort. De drie musketiers grijpen elkaar bij de gesjerpte schouders en dansen heel stijlvol de Griekse zirtaki....

Ik besluit me in het donker van het balkon terug te trekken om ongestoord goed naar de andere kant van de zaal te kunnen kijken. Er is zoveel leuks te zien.

In de gezellig grote ruimte bevinden zich 3 grote wenteltrappen die in verbinding staan met de hogere gelegen balkon-delen. Een wat kleinere gestalte daalt één van de wenteltrappen af, duidelijk een man, heel zijn houding straalt kracht, autoriteit en plezier uit. Het is overduidelijk Jaap, dé Jaap, professor Jaap Zwartendijk, uroloog in het LUMC, Leiden. Een leermeester pur sang. Wentel, de bocht om. Een grijns verlicht zijn gezicht. Iedereen weet dat deze man met een stoere goedheid en een onverschrokken beheerst moreel zijn gelederen genadeloos temt en opvoedt in rechtvaardigheid. Dat is nou Jaap, hij weet alles en is overal. Op dat moment grijnst hij naar me en trekt zijn linker wenkbrauw hoog, heel hoog op terwijl de rechter wenkbrauw laag blijft. De sjerp past trouwens goed bij zijn lintje. Ik wil zwaaien en "joehoeoe" roepen en ".....zolang het maar klinisch relevant en niet te duur is, hè Jaap!" maar hij draait de wentel weer door. Ik zie hem richting de ronde tafels die voor het podium staan lopen. Daar zit ik ook professor Schröder, uroloog, herr Fritz, dat laatste zou ik trouwens nooit tegen hem durven zeggen, maar ik vind hem zo bijzonder. Met rood strikje en geknepen ogen zit hij samen met Chris Bangma, professor Chris Bangma, aan een tafeltje vlak naast het podium, ze lachen heel ontspannen. Een slanke vrouw met halflang zwart haar zweeft sierlijk langs, och, het is die lieve, pittige mevrouw Bonnema. Ik herinner me de gezellige etentjes en bijeenkomsten met de prostaatgroep. Helaas spraken we natuurlijk veel te veel over prostaatscreening, à la, niet erg Ingrid kwam ervoor terug uit Zwolle. Oh kijk Winand & Wil, Wil & Winand zijn er ook, dan is het daar zeker gezellig. Professor M Hunink en professor W Oosterhuis zie ik daar ook zitten. Professor B Kiemeney schuift aan en er lijkt zich een geanimeerd gesprek te ontwikkelen.

Ondertussen deint de dansvloer op de ritmische golven van het combo bandje, dat zich inmiddels versterkt ziet met een aantal musici. Met een Bas-gitarist die de lead ondersteund door vol overgave de hendel op zijn gitaar heen en weer te bewegen (lijkt trouwens erg op Bas van Rhijn, och ... het ís Bas van Rhijn), met dé Theo van der Poel die met zijn mondharmonica heel knap een bridge maakt naar het nummer 'money, money, money' en daarnaast ook nog een verrassend soepele moonwalk uit de kast trekt. Op de banjo zet Marie-Louise Essink Bot een harmonische septiem in. Twee achtergrondzangeressen worden aangekondigd als de typical ladies of '100 swings a minute' Carrie en de enige echte Ingrid van Woerkoóóóm. De zaal barst uit zijn voegen als beide dames knippend met de vingers en draaiend met de heupen een kraakhelder 'ahoe' ten gehore brengen. Een daverende drumriff van niemand minder dan Irene Lurkin dwingt de zaal weer tot beweging. En dan in een volle octaaf hoger, in een perfecte harmonie, zingen Marion Verhoef, Fatima en Mina het kippenvel op ieders armen, wat een warme stemmen.... ondertussen worden er steeds mensen het podium opgeroepen en door de bekende cabaratier met pakkende woorden en een CEFUB-sjerp gehuldigd. ZON-MW heeft dat gratis weten te regelen.

In de verte zie ik in een loungehoek onder een balkonnetje een gehuldigd groepje gebogen staan over een laag tafeltje. Nieuwsgierig ga ik even kijken. Het zijn Merel, Kirstin, Yvette en Twan. Ze spelen een analytisch spel. Het ziet er razend moeilijk uit maar ze schijnen exact te weten wat ze moeten doen. Om hun heen staan Angela, Marcel, Magda, Tahlita, Martijn en Marjolein. Af en toe mogen deze omstanders ook wat roepen of joelen. Bij verlies van een potje moet je blijkbaar een hoeveelheid grote glazen epjes met een gelige vloeistof opdrinken. Dat gaat dan gepaard met een afgrijzend ge-oe door de rest van de groep en de omstanders, een teken voor Irene-Lurkin-achter-drums om prompt een drumsalvo af te vuren. Deze club is ontzettend belangrijk geweest voor de CEFUB-trial.

Rondom de favorite punch-bar waarop bier, zoete-witte-wijn opties en hot chicken wings staan een aantal rare lui volledig uit hun dak te gaan. De één nog gekker dan de ander. Een man met snor komt naar me toe en ik schrik. Omdat ik hem niet meteen herken wrijft hij door zijn krullen en steekt een sjakkie op! Vervolgens doet hij zijn snor omhoog en bromt vanuit de grillige diepten van zijn rokerige longen: "...It is I, Alèx!.." Hé Aaaaaahl roepen 2 stemmen in koor vlak achter me, het zijn dé Moniqueskes. Twee blonde Brabantse moeders met veel jolijt in de kop. Ik geniet, daar komt ook Brutus aandansen. Met een uitgestreken gezicht, waarop een veel te grote zware zwarte zonnebril, swingt hij vol overgave recht op het chicken wings rek af. Alex ziet dat en geeft hem een klein corrigerend duwtje richting de punch.....en gromt "dat spul is toch niet te zuipen." Ik wil graag blijven maar dan zie ik een aantal dames lachend discussieren. Het zijn Hilde, Esther, Lucie, Joke, Fatma en Renske. Lieve mensen die me dierbaar zijn in vriendschap en wetenschap en prachtige resultaten hebben beschreven. Mijn aandacht wordt getrokken naar een lawaaierig balkon en ik sla Lucie en

Fatma nog met een harde klop op de schouders en knijp nog even lekker in hun wangen.... zet 'm op lieve ladies!

Op een hoog balkon staan een aantal mensen op geheel eigen en subtiele wijze met hun wijsvinger en met scopes in de lucht te priemen en te zwaaien. Volle bak op dat balkon. Je ziet kalende, blonde, kale, extreem blonde, gekrulde en hele kort gestylde koppies, ontzettend gebruinde en iets blekere gezichten, groene en blauwe kapjes, met en zonder bril en er klinkt een enorm gekakel. De wijn en de lach vloeien rijkelijk, de kaas ruikt lekker. Wat een gezellige groep. Daar Henk en Milou, een veelbelovende energieke combinatie. Dat zijn nou de urologie collega's van het LUMC en het Haga en oh Ruben staat er ook (natuurlijk met twee wijsvingers) te priemen en te shaken ....joehoeoe... wat een gezelligheid!

Als ik heel ver voorover leun en over de rand van de ballustrade hang kan ik door de ingang van de zaal de grote entrée hal inkijken en de foyer zien. Aan de bar in de foyer van het gebouw staat een groep mensen luid te lachen, kinderen dansen er omheen en spelen verstoppertje. Dat was lang geleden dat ik die zo met zijn allen heb zien staan. Suus, Reinout en Lars, Ivo, Milou, Sas, Rem en Lotte, Olivier, Merel, Iris, Eric en Benthe, Mickel, en nog een heel klein wezentje, Manon, Martijn en Julius, Joyce & Martijn, Auk, Tone en Finn, Ollie, Alain en Max, Tessa, Lionel en Coco, Simone, Deau en Joep, Guus, Jack, Jor en Mees, Fiep, Floor, Ilse, Peter en Klaas, Adriaan, Koen, Caroline en Lucas, Liesbeth, Herman, Walter, Severine en Jort, Olivier, Nicky, Hans en Ivy, Fenn. Tot snel! En ik leun nog verder naar voren om alle anderen te kunnen zien en te roepen dat het te lang geleden is en dat het me spijt dat het zo lang geleden is en dat ik slecht bereikbaar was enzo.

Maar dan word ik ineens aan mijn benen naar beneden getrokken. Het is de familie Hornstra. Ze hebben mijn benen weer op de grond gezet. 'Dat was gevaarlijk hoor,' zegt Anniek lief en Floris doet meteen op de grond voor wat er had kunnen gebeuren als ze me niet hadden vastgepakt en roept hard lachend 'farligt!' Peet en Niels! Ik vlieg ze om de hals, wat ben ik blij om ze weer te zien. Belangeloos en menselijk, het gewoonste en meest wonderlijke, eerlijk en altijd geduldig, echte vrienden zijn het. Ook Jennie, Jeroen, Valentijn en Julius staan vlak naast me en kijken mee de zaal in. Valentijn en Julius hebben, liggend op hun buik, hun hoofden over de rand gehangen en kijken wie er het verst kan spugen. Valentijn biedt, genereus als hij is, Lulies nog een verzwaard spuugje aan, maar hij heeft er al één en rochelt een beste de menigte in. Jeroen ziet het en grijpt in. En kleine mama Jennie groeit, mijn Sjaan is zichtbaar trots op haar drie mannen. Met een volledig ontwikkelt mediteraan gevoel van moederschap en ware vriendschap danst zij door het leven. Wat een plezier om mee door het leven te dansen, onvergelijkbaar en onvergetelijk. Valentijn volgt ondertussen een spoor van stro, met in zijn kielzog een wankele Julius, dat leidt naar een hoogst eigenaardig tafereel. Daar staat een shetlander met een pluizig mupje midden op haar kop, een fors konijn zo te

zien. Ze snorkt tegen een IJslander aan waarop een heel klein pittig meisje haar flesje drinkt. Bízar! En het lijkt ook nog allemaal met elkaar te communiceren. Wat blijkt: Viktor zit op de kop van Heidi en fluistert haar in het oor dat ze aan Blökk moet vragen of hij bij Britt mag zitten, omdat hij Britt zo lief vindt. De vader van Britt staat er naast, Niels! Met een hele grote glimlach houdt hij haar met één hand vast. Maar dat betekent maar één ding, mijn Roomie is in de buurt, Roooomiiie! Waar is ze? Niels wijst met zijn vrije hand en een nog grotere glimlach naar de onderkant van het paard. Als ik dan tussen het stro mijn lieve Roomie zie, wisselen we toch snel even de meest fantastische grappen uit. Ze knielt achter de uiers van Blökk, stro in haar haar en in haar mond. Roomie!?! Wat...wat doe je daar? Terwijl ze onder Blökk vandaan kruipt, houdt ze haar handen heel voorzichtig als een kommetje. Ze straalt. Ik lach. Ze krabbelt moeilijk op, ik lach en geef haar een zacht duwtje, ze sluit haar handen en lacht. Mysterieus loopt ze naar Britt, geeft Niels een kus en stopt iets bij Britt in haar zakjes, mijn hart glimlacht. Britt straalt. Linetta en Niels geven licht. Het liefst zou ik het hoofd van Roomie tussen mijn handen nemen om te vertellen hoe mooi en bijzonder ze is, maar ik weet het even niet goed uit te drukken, het wonder van een oprechte vriendschap die ooit begon in Be-332 en alleen maar groeit.

Rondom de paarden staat de familie Lubbers te kletsen en te lachen met elkaar en iedereen. Janne Sophie en Florian houden de paarden aan een touw, Manon en Herman vertellen de kinderen over 'Viktor en de Ooievaar', Pauline loopt rond met een dienblad vol heerlijke hapjes (hoe doet ze dat toch?) terwijl Jan Harman een strootje uit het haar van Linetta plukt en, aan wie het maar horen wil, vertelt over de ontwikkeling van zijn laatste project, 'stro en kastanjes, heel 2009'. En daartussen staan lieve Leo en Margareth te genieten van het samenzijn, de open haard brandt en het knetterend haardvuur voelt als thuis.

Broertjes! Suusjes! Ook hier! Lieve Sjoerd & Suus & Maurits, Sytze & kleine Suus & lief klein mensje in de buik van kleine Suus. Hier, ooit, daar, nu, toen, aan tafel, ontbijt, diner of late-night snack, in een kroeg met of zonder dak, aan de telefoon of op het veld, overal is thuis met jullie. Proooost, zeggen we heel hard en ik kijk even trots naar Roos en Ton, naar míjn lieve moeder en vader, zij zitten zoals altijd in een hele comfortabele chaise longue op mijn linker schouder. We steken de duimen omhoog en ik voel de kracht en de warmte diep in mijn ziel en mijn hart maakt een huppeltje.

Dan voel ik op mijn rechter schouder een hand heel even liefdevol een zacht kneepje geven. Die sterke hand, zo bekend en betrouwbaar. Mijn gezicht gloeit als ik me omdraai en recht in zijn warme ogen kijk. Mijn grote liefde. Op zijn rechter arm draagt hij ons klein lief mannetje. Het kleine wezentje lacht, en lacht de liefste en warmste glimlach van de wereld....kom we gaan! Op naar een nieuw avontuur....samen!

Participants in CEFUB-trial

Sint Franciscus Gasthuis, Rotterdam	Drs. ER Boevé, Department of Urology Dr JHM Blom, Department of Urology Dr J Rietbergen, Department of Urology Dr JA Ruizeveld de Winter, Department of Pathology
Havenziekenhuis, Rotterdam	Dr SM Haensel, Department of Urology Drs. ER Boevé, Department of Urology
Ikazia Ziekenhuis, Rotterdam	Drs. E Kats, Department of Urology Dr EJ Nienhuis, Department of Urology Drs. JK Boldewijn, Department of Pathology
Vlietland Ziekenhuis, Locatie Schieland Schiedam	Dr IL Lie, Department of Urology
Maasland Ziekenhuis, voorheen MCRZ Locatie Clara, Rotterdam	Dr D van den Ouden, Department of Urology Drs. JK Boldewijn, Department of Pathology
Reinier de Graaff Groep, S.S.D.Z, Delft	Dr JFAT Bogdanovicz, Department of Urology Dr C van Krimpen, Department of Pathology
Albert Schweitzer Ziekenhuizen, Dordrecht Locatie Dordwijk, Locatie Sliedrecht Locatie Zwijndrecht	Drs. AGM Zeegers, Department of Urology Drs. J Vis, Department of Urology Drs. PA Wertheimer, Department of Urology Drs. WM Stomps, Department of Urology Drs. RM Potjer, Department of Urology Dr M Kuizinga, Department of Pathology
Amphia Ziekenhuizen, Breda / Oosterhout Locatie Molengracht Locatie Langendijk Locatie Pasteurlaan	Drs. EHGM Oomens, Department of Urology Drs. H Jansen, Department of Urology Drs. DKE Van der Schoot, Department of Urology Drs. PJ Posthumus, Department of Urology Dr TEG Ruijter, Department of Pathology

Leids University Medical Center	Prof J Zwartendijk, Department of Urology
Leiden	Prof Dr AAB Lycklama à Nijeholt, Department of Urology
	Dr RCM Pelger, Department of Urology
	Dr RF Bevers, Department of Urology
	Dr HW Elzevier, Department of Urology
	C de Jong-Mom, Department of Urology
	Prof Dr H Morreau, Department of Pathology
Erasmus MC, Rotterdam	Prof dr Th H van der Kwast, Department of Pathology
	Prof dr EW Steyerberg, Department of Public Health
	Prof dr EC Zwarthoff, Department of Pathology
	Prof dr CH Bangma, Department of Urology
	Dr M-L Essink-Bot, Department of Public Health
	Drs. EF Sen, Department of Epidemiology
	Drs. EW de Bekker-Grob, Department of Public Health
	Dr MJC Eijkemans, Department of Public Health
	Dr BWG van Rhijn, Department of Urology
	Dr WJ Kirkels, Department of Urology
	Ing M Verwey, Department of Pathology
	Ing I Lurkin, Department of Pathology
	Ing MW Boogaard, Department of Pathology
	Ing Y Nijssen, Department of Pathology
	Ing KA van der Keur, Department of Pathology
	Ing AJA van Exsel, Department of Pathology
	Drs. Th van der Poel, Department of Pathology

Contributing Authors

Prof dr. CH Bangma
Department of Urology
Erasmus MC
Rotterdam

Drs. EW de Bekker-Grob
Department of Public Health
Erasmus MC
Rotterdam

Drs. MD Bekker
Department of Urology
Leids University Medical Center
Leiden

MW Boogaard, Y Nijssen, KA van der Keur,
AJA van Exsel, I Lurkin
Department of Pathology
Erasmus MC, Rotterdam

Dr. A Brinkmann
Institute of Pathology
University of Regensburg
Regensburg, Germany

Dr. M Burger
Department of Urology
University of Regensburg
Regensburg, Germany

Dr. S Denziger
Department of Urology
University of Regensburg
Regensburg, Germany

Drs. M Dönmez
Department of Public Health
Erasmus MC
Rotterdam

MJC Eijkemans
Department of Public Health
Erasmus MC
Rotterdam

Dr. HW Elzevier
Department of Urology and Sexuology
Leids University Medical Center
Leiden

Dr. M-L Essink-Bot
Department of Public Health
Academic Medical Center at the University
of Amsterdam

Prof dr. A Hartmann
Institute of Pathology
University Erlangen
Erlangen, Germany

Dr. F Hofstaedter
Institute of Pathology
University of Regensburg
Regensburg, Germany

Dr. AC Jöbsis
Department of Pathology
Sint Franciscus Gasthuis / LUMC
Rotterdam / Leiden

Dr. WJ Kirkels
Department of Urology
Erasmus MC
Rotterdam

Prof dr. Th H van der Kwast
Department of Pathology
University Health Network
Toronto, Canada

Dr. GJLH van Leenders
Department of Pathology
Erasmus MC
Rotterdam

Dr. JMM van Oers
Department of Cell Biology
Albert Einstein College of Medicine
Bronx, New York, USA

Dr. BWG van Rhijn
Department of Urology
University Health Network
Toronto, Canada

Drs. EF Sen
Department of Epidemiology
Erasmus MC
Rotterdam

Prof dr. EW Steyerberg
Department of Public Health
Erasmus MC
Rotterdam

Dr. R Stoehr
Institute of Pathology
University Erlangen
Erlangen, Germany

Dr. WF Wieland
Department of Urology
University of Regensburg
Regensburg, Germany

Dr. PJ Wild
Institute of Surgical Pathology
University Hospital Zurich
Zurich, Switserland

Prof dr. EC Zwarthoff
Department of Pathology
Erasmus MC
Rotterdam

List of Publications

1. **Madelon N.M. van der Aa**, Theo H. van der Kwast, Ellen C. Zwarthoff.
   Toepassing van moleculaire diagnostiek bij de follow-up van patiënten met oppervlakkig blaascarcinoom, een onderzoek naar de kosten-effectiviteit.
   Tijdschrift Kanker Oktober 2002, p12-14
2. **van der Aa MN**, van Leenders GJ, Steyerberg EW, van Rhijn BW, Jobsis AC, Zwarthoff EC, van der Kwast TH.
   A new system for substaging pT1 papillary bladder cancer: a prognostic evaluation.
   Hum Pathol. 2005 Sep;36(9):981-6.
3. Mongiat-Artus P, Miquel C, **van der Aa M**, Buhard O, Hamelin R, Soliman H, Bangma C, Janin A, Teillac P, van der Kwast T, Praz F.
   Analysis of the microsatellite instability gene mutation in the urothelial tumour of the upper urinary tract
   Prog Urol. 2005 Dec;15 (6 Suppl 1):1286-92. French
4. van Oers JM, Lurkin I, van Exsel AJ, Nijsen Y, van Rhijn BW, **van der Aa MN**, Zwarthoff EC.
   A simple and fast method for the simultaneous detection of nine fibroblast growth factor receptor 3 mutations in bladder cancer and voided urine
   Clin Cancer Res. 2005 Nov 1;11(21):7743-8.
5. Mongiat-Artus P, Miquel C, **van der Aa M**, Buhard O, Hamelin R, Bangma C, Teillac P, van der Kwast T, Praz F.
   Infrequent microsatellite instability in urothelial cell carcinoma of the bladder in young patients
   Eur Urol. 2006 Apr;49(4):685-90. Epub 2006 Jan 4.
6. Mongiat-Artus P, Miquel C, **Van der Aa M**, Buhard O, Hamelin R, Soliman H, Bangma C, Janin A, Teillac P, van der Kwast T, Praz F.
   Microsatellite instability and mutation analysis of candidate genes in urothelial cell carcinomas of upper urinary tract
   Oncogene. 2006 Mar 30;25(14):2113-8.
7. A. van Geel, M. den Bakker, W. Kirkels, S. Horenblas, B. Kroon, J. de Wilt, A. Eggermont, W. Mooi, **M. van der Aa**.
   Prognosis of primary mucosal penile melanoma: a series of 19 Dutch patients and 47 patients from the literature.
   J of Urology. 2007 Jul;70(1):143-7. Review
8. **Madelon N.M. van der Aa**, Ewout W. Steyerberg, E. Fatma Sen, Ellen C. Zwarthoff, Wim J. Kirkels, Theo H. van der Kwast and Marie-Louise Essink-Bot.
   Patients' perceived burden of cystoscopic and urinary surveillance of bladder cancer: a randomized comparison
   British Journal of Urology Int., 2008 May; 101(9): 1106-10. Epub 2007 Sept 20

9. Ellen C. Zwarthoff, **Madelon N.M. van der Aa**, Tahlita C.M. Zuiverloon, Lucie Kompier, Ewout W. Steyerberg, Wim J. Kirkels, Chris Bangma, Theo H. van der Kwast.
   Blaaskanker: een terugkerend probleem.
   Tijdschrift Kanker Juni 2008, p16-19.
10. **van der Aa MNM***, Burger M*, van Oers JMM, Brinkmann A, van der Kwast ThH, Steyerberg EC, Stoehr R, Denzinger S, Wild PJ, Wieland WF , Hofstaedter F, Hartmann A, Zwarthoff EC.
    Prediction of Progression of Non-Muscle-Invasive Bladder Cancer by WHO 1973 and 2004 Grading and by FGFR3 Mutation Status: A Prospective Study
    European Urology, 2008 Oct; 54(4): 835-43. Epub 2007 Dec 26
11. **Madelon N.M. van der Aa**, Ellen C. Zwarthoff, Ewout W. Steyerberg, Merel W. Boogaard, Yvette Nijsen, Kirstin A van der Keur, Antonius J. A. van Exsel, Wim J. Kirkels, Chris Bangma, Theo H. van der Kwast.
    Microsatellite Analysis of Voided-Urine Samples for Surveillance of Low-Grade Non Muscle-Invasive Urothelial Carcinoma: Feasibility and Clinical Utility in a Prospective Multicenter Study (Cost-Effectiveness of Follow-Up of Urinary Bladder Cancer Trial -CEFUB]).
    European Urology, Epub 2008 May 15
12. Esther W. de Bekker-Grob, **Madelon N.M. van der Aa**, Ellen C. Zwarthoff, Marinus J.C. Eijkemans, Bas W. van Rhijn, Theo H. van der Kwast, Ewout W. Steyerberg. Cost-effectiveness of non-muscle invasive bladder cancer surveillance in which cystoscopy is partly replaced by microsatellite analysis on urine (CEFUB-trial)
    British journal of Urology international, accepted for publication October 2008.
13. **Madelon NM van der Aa**, Milou D Bekker, Fatma EF Sen, Theo H van der Kwast, Marie-Louise Essink-Bot, Ewout W Steyerberg, Ellen C Zwarthoff and Henk W Elzevier.
    Sexual function of patients under surveillance for bladder cancer
    British Journal of Urology Int., accepted for publication October 2008.
14. **Madelon N.M. van der Aa**, Ewout W. Steyerberg, Chris Bangma, Bas W.G. van Rhijn, Ellen C. Zwarthoff and Theo H. van der Kwast.
    Cystoscopy revisited as the gold standard for detection of bladder cancer recurrences: diagnostic review bias in a randomized prospective trial (CEFuB-trial)
    Submitted.
15. Lucie C. Kompier*, **Madelon N.M. van der Aa***, Irene Lurkin, Marcel Vermeij, Wim J. Kirkels, Chris H. Bangma, Theodorus H. van der Kwast and Ellen C. Zwarthoff.
    The development of multiple bladder tumour recurrences in relation to the FGFR3 mutation status of the primary tumour
    Accepted for publication, dec 2008.
16. **Madelon N.M. van der Aa***, Mustafa Dönmez*, Marinus JC Eijkemans, Theo H. van der Kwast, Ellen C. Zwarthoff and Ewout W. Steyerberg.

Clinical and Pathological Prognostic Factors for Recurrence, Progression and Mortality in non-muscle invasive Bladder Cancer: a Meta-analysis
Submitted.

17. Tahlita C.M. Zuiverloon, **Madelon N.M. van der Aa**, Theo H. van der Kwast, Ewout W. Steyerberg, Chris H. Bangma, Ellen C. Zwarthoff.
FGFR3 mutation status on voided urine as a strong predictive tool for surveillance of patients with non-muscle invasive bladder cancer
Submitted.
18. **Madelon N.M. van der Aa**, Theo H. van der Kwast, E. Fatma Sen, Ellen C. Zwarthoff, Marie-Louise Essink-Bot and Ewout W. Steyerberg.
Distress in patients with bladder cancer, a longitudinal study
Submitted.