Seeing upper tract urothelial carcinoma in a new light

Freund, J.E.

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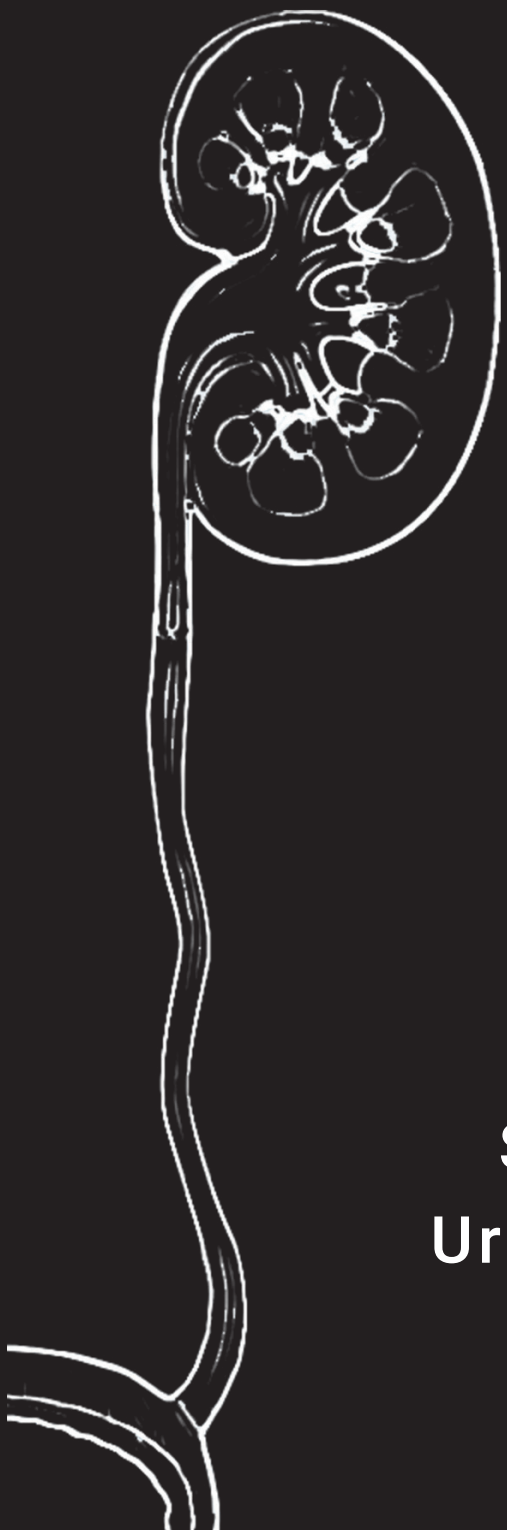

J.E. Freund

Seeing Upper Tract Urothelial Carcinoma in a New Light

Seeing
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in a New Light

Jan Erik Freund

Seeing
Upper Tract Urothelial Carcinoma in a New Light

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Chapter 1

General introduction and outline of the thesis

List of abbreviations

CLE – confocal laser endomicroscopy
CIS – carcinoma in situ
EAU – European Association of Urology
OCT – optical coherence tomography
TNM – tumour node metastasis
UCB – urothelial carcinoma of the bladder
UTUC – upper tract urothelial carcinoma
WHO – World Health Organization

Urothelial carcinoma in the urinary tract

The urinary tract is divided into the lower and upper urinary tract. While the lower urinary tract consists of the bladder and the urethra, the upper urinary tract is composed of both ureters and renal pelves. The lower and the upper urinary tract are covered by mucosa, called urothelium. Healthy urothelium consists of two to seven layers of urothelial cells that lie on top of the lamina propria. The lamina propria is a layer of connective tissue that separates the urothelium from the underlying muscle, called the muscularis propria.

Malignancies of the urinary tract arise almost exclusively from the urothelium, and are thus called urothelial carcinoma. The histopathology of urothelial carcinoma of the bladder (UCB) and upper urinary tract are alike, but the diseases are considered 'disparate twins'.[1] Up to 41% of patients with upper tract urothelial carcinoma (UTUC) have a prior medical history of UCB. [2] Moreover, concurrent UCB is identified at the time of initial UTUC diagnosis in up to 17% of patients.[3] UTUC, however, is less prevalent compared to UCB. UTUC contributes only 5-10% to the sum of urothelial carcinomas. In Western countries, UTUC has an annual incidence of 2 cases per 100.000 people, but an increasing incidence has been reported.[4,5] UTUC is seen three times more often in men than in women.[6] The highest incidence is found in the population of 70-90 years of age. Yet, UTUC may also occur in younger people as it can be related to hereditary nonpolyposis colorectal carcinoma, called Lynch syndrome.[7]

Histopathology

Urothelial lesions can be divided into two distinct groups based on the cellular architecture: papillary tumours and flat lesions. Papillary UTUC exhibits a papillary growth pattern, in which the malignant cells are arranged around central fibrovascular cores, resulting in a finger-like appearance (Figure 1). As defined by the International Society of Urological Pathology and the classification of the World Health Organization (WHO) 2004/2016, papillary UTUC is furthermore divided by its histopathologic grade into low-grade and high-grade disease.[8–10] Histopathologic grading is based on the degree of nuclear atypia and cellular disorganization (Figure 1). Flat lesions are devoid of papillary structures and are called carcinoma in situ (CIS), which by definition imply a high-grade lesion. CIS may progress to invasive UTUC, and is, therefore, considered a high-risk disease.[8]

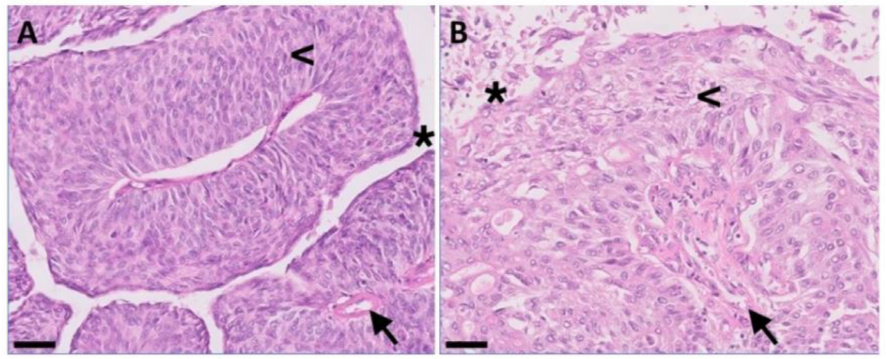


Figure 1: histopathologic H&E images of papillary upper tract urothelial carcinomas. The scale bar represents 50 µm. A) low-grade urothelial carcinoma with mild cytonuclear atypia, fibrovascular core (↖), preserved cellular cohesiveness (*), preserved polarity and cellular organization (<). B) high-grade urothelial carcinoma with high cytonuclear atypia, fibrovascular core (↗) loss of cellular cohesiveness (*), loss of polarity and cellular organization (<).

Besides the histopathologic grade, the histopathologic stage of the tumour is also an important tumour characteristic. Histopathologic staging is based on the extent of tissue invasion (Figure 2).[11] The histopathologic grade and stage are the key prognostic factors of UTUC.

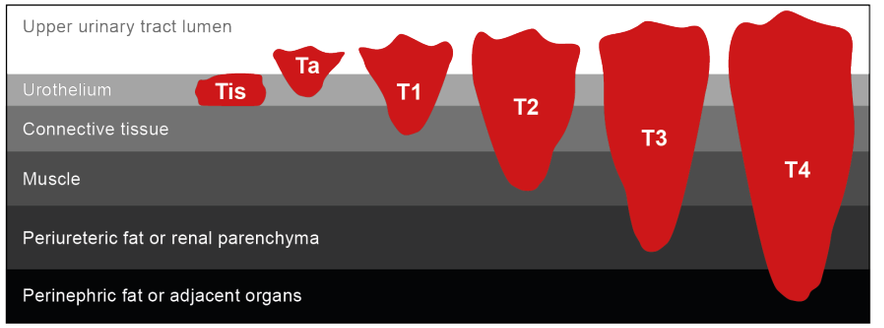
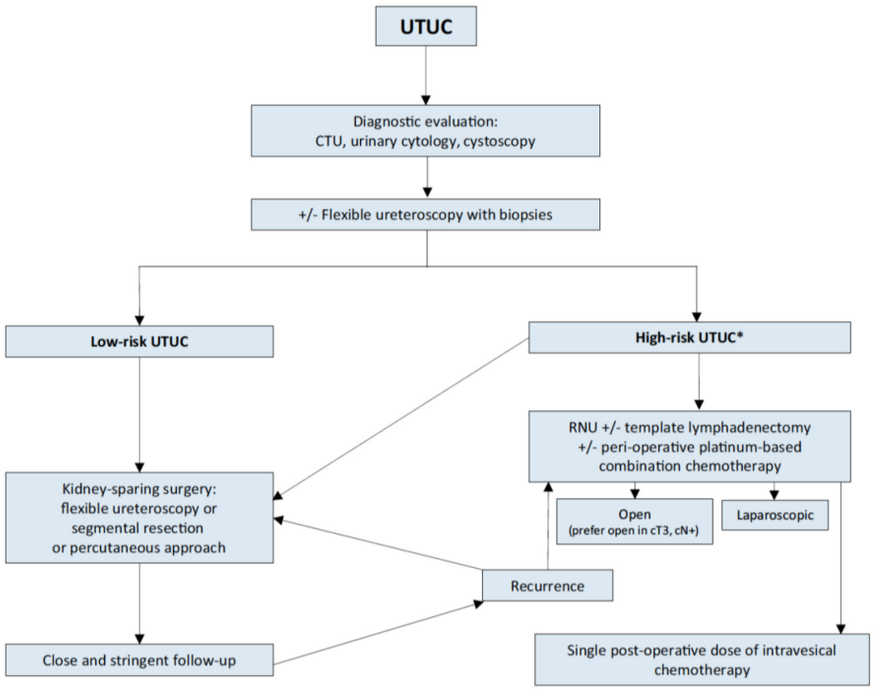


Figure 2: histopathologic stages of upper tract urothelial carcinoma (UTUC) according to the 8th edition of the TNM classification; carcinoma in situ (Tis), non-invasive UTUC (Ta), UTUC with invasion of the subepithelial connective tissue (T1), UTUC with muscle invasion (T2), UTUC with invasion beyond the muscle into periureteric fat or renal parenchyma (T3), UTUC invading through the kidney into perinephric fat or adjacent organs (T4).

The diagnostic paradigm of upper tract urothelial carcinoma

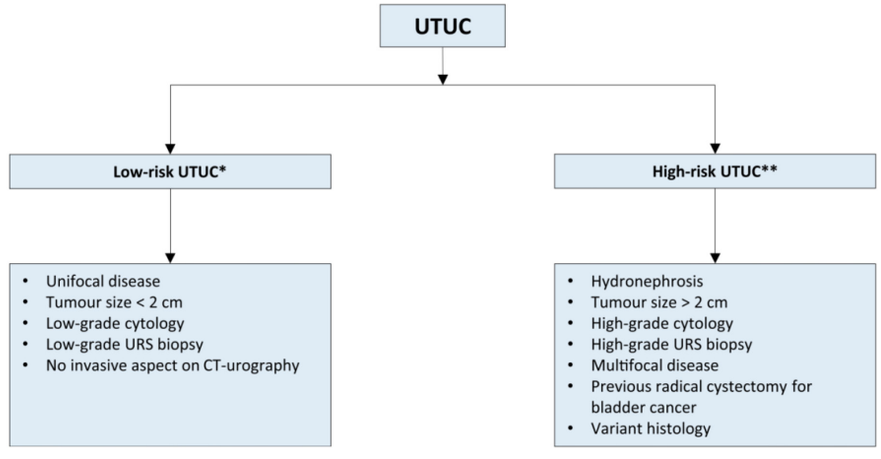
Radiologic imaging is used to identify suspicious lesions in the upper urinary tract in the event a patient presents with symptoms e.g. haematuria or flank pain. Also, cytologic assessment of urine, especially selectively collected from the upper urinary tract, may be used as an initial diagnostic test to rule out the presence of high-grade UTUC and CIS. The diagnostic value of urinalysis may be increased with cytogenetic testing such as UroVysion® fluorescence in situ hybridization, which allows for the visualization of molecular alterations of urothelial carcinoma.[12–14] If radiologic imaging and cytology do not provide diagnostic certainty, especially if kidney-sparing treatment is considered, flexible ureterorenoscopy with biopsies are essential to the diagnostic paradigm, as illustrated by the European Urology Association (EAU) guidelines (Figure 3).[9]



**In patients with solitary kidney, consider a more conservative approach.*
CTU = computed tomography urography; RNU = radical nephroureterectomy;
UTUC = upper urinary tract urothelial carcinoma.

Figure 3: proposed flowchart by the EAU guidelines 2020
for the management of upper tract urothelial carcinoma.

Flexible ureterorenoscopy is an endoscopic procedure that is usually performed under general anaesthesia. By making use of the existing anatomic structures, ureterorenoscopy is considered a minimally invasive technique. To reach the upper urinary tract, the ureterorenoscope is introduced via the urethra and the bladder into either ureteral orifice. Owing to the miniaturization and the flexibility of scopes, every part of the upper urinary tract can be reached by flexible ureterorenoscopy. By visualization of the complete urothelial lining of the upper tract, ureterorenoscopy enables the identification of papillary UTUC. In contrast, CIS is less readily identifiable, owing to its non-specific flat architecture. The working channel of ureterorenoscopes enables the introduction of a biopsy forceps or similar instruments to harvest tissue biopsies for histopathologic assessment. Ureterorenoscopic tissue biopsies permit the initial histopathologic diagnosis of the identified lesion in the upper urinary tract. In case of UTUC diagnosis, these biopsies also facilitate the assessment of the histopathologic grade. As already mentioned, the histopathologic grade is a key prognostic factor. Amongst other variables, the histopathologic grade is essential to the risk-stratification of the disease, in which UTUC is divided into low-risk and high-risk disease (Figure 4). This risk stratification is recommended by the EAU guidelines with the aim to warrant optimal oncologic outcomes by providing an individualized approach for treatment selection (Figure 3).[9,15]



CTU = computed tomography urography; URS = ureteroscopy; UTUC = upper urinary tract urothelial carcinoma.
*All these factors need to be present.
**Any of these factors need to be present.

Figure 4: recommendation of the EAU guidelines 2020
for the risk stratification of upper tract urothelial carcinoma.

Treatment of upper tract urothelial carcinoma

High-risk UTUC is generally treated by radical nephroureterectomy. This means surgical resection of the complete kidney, ureter and the ipsilateral bladder cuff. As a result, this surgical approach could lead to renal insufficiency and dialysis.

Alternatively, UTUC can be treated by kidney sparing approach, e.g. segmental ureterectomy or ureterorenoscopic laser ablation. Segmental ureterectomy can be performed if the tumour is located in the distal ureter. In segmental ureterectomy, the diseased part of the ureter is removed with a reconnection of the remaining ureter to the bladder by e.g. a psoas hitch. Alternatively, ureterorenoscopic laser ablation can serve as a treatment in which the pre-existing anatomy and renal function is preserved. For ureterorenoscopic laser ablation, a laser fiber is introduced via the working channel of the ureteroscope. The laser energy applied is used to ablate the tumour from within the upper urinary tract during ureterorenoscopy.

Ureterorenoscopic laser ablation was initially offered to imperative cases only, i.e. patients with renal insufficiency or solitary kidneys. However, on the grounds of the similar oncologic outcome of laser ablation in comparison to radical nephroureterectomy in low-risk UTUC, ureterorenoscopic laser ablation has become increasingly popular in low-risk disease.[16] As a result, the risk-stratification of the disease has become essential to the diagnostic paradigm to warrant good clinical practice.

On the downside, high local recurrence rates of up to 51% in the treated upper urinary tract are reported after ureterorenoscopic laser ablation.[17,18] In consequence, kidney sparing surgery is followed by a highly burdening and indefinite ureterorenoscopic follow-up of the treated upper urinary tract.[9,18]

Shortcomings of the current diagnostic paradigm

Despite the crucial role of ureterorenoscopic biopsies in the diagnostic paradigm of UTUC, tissue sampling may be hampered by the restricted range of motion in the narrow anatomy of the upper urinary tract and the tangential approach of suspicious lesions. Consequently, unrepresentative (i.e. not the tissue of interest) or insufficient tissue sampling (i.e. insufficient tissue or tissue of insufficient quality for reliable histopathologic assessment) result in limitations of the diagnostic yield and the diagnostic accuracy of ureterorenoscopic biopsies in the diagnostic approach to UTUC.[19–26] In the literature, the diagnostic yield of ureterorenoscopic biopsies for UTUC grading ranges extensively with reported results of 65% to 100%. Moreover, inaccurate grading of UTUC has been reported in up to 51% of biopsies.[20] Histopathologic staging in ureterorenoscopic biopsies is even less reliable because ureterorenoscopic biopsies are generally too superficial.[9,19,27] However, nationwide cohort studies, evaluating the abovementioned issues, are still lacking.

Another shortcoming of the current diagnostic approach is the inability to assess the histopathologic grade during ureterorenoscopy in real-time. The prediction of histopathologic grade based on the visual appearance of UTUC during fiber optic ureterorenoscopy is inaccurate.[28] Studies on the diagnostic accuracy of grade predictions during digital ureterorenoscopy are lacking, and such predictions are not advocated in any international guideline.

Moreover, the risk of complications of ureterorenoscopic biopsies must be considered. In the literature, more severe, but infrequent complications such as ureter perforation or ureteral stripping have been reported.[29] Bleeding is observed more frequently, but is generally mild. Blood transfusions are seldomly required, but bleeding can impair the endoscopic vision. Impaired endoscopic vision affects the effectiveness and potentially the safety of immediate laser ablation after ureterorenoscopic biopsies.

From the abovementioned shortcomings, it follows that some patients, who qualify for kidney-sparing treatment according to one of the criteria recommended for risk-stratification, might be stratified incorrectly. Furthermore, the efficiency and safety of the current diagnostic approach could be improved. As a result, we should seek new ways to distinguish low-grade from high-grade UTUC for improvements in oncologic outcomes.

Emerging optical imaging modalities such as optical coherence tomography (OCT) and confocal laser endomicroscopy (CLE) may hold the potential to overcome the diagnostic challenges at hand.[30] So far, first promising results have been reported for optical coherence tomography (OCT) and confocal laser endomicroscopy (CLE).[31,32] Both techniques are probe-based optical imaging techniques that are compatible with the working channel of flexible ureterorenoscopes. These optical imaging techniques enable high resolution imaging of the tissue of interest during ureterorenoscopy. Optical biopsies may potentially replace ureterorenoscopic tissue biopsies, and therewith overcome the shortcomings of the current diagnostic paradigm. Nonetheless, further clinical investigations are required before these techniques may be implemented in clinical care.

General aims of this thesis

The aims of this thesis are:
I.Understanding the shortcomings of the current diagnostic approach of UTUC.
II.Investigating the diagnostic potential of novel optical techniques for UTUC.

Outline of this thesis

Chapter 2 provides a retrospective, nationwide, cohort study that explores the diagnostic yield and concordance of histopathologic grading between ureterorenoscopic biopsies and surgical resections of UTUC in the Netherlands. The study cohort was based on pathology excerpts of the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA) from 2011 until 2018.

In **Chapter 3**, the limitations of grade predictions based on the visual appearance of papillary UTUC during digital ureterorenoscopy are described with regard to the diagnostic accuracy, the intra-, and inter-rater agreement.

Chapter 4 summarises the state of the art of OCT in urologic oncology. Besides the technical aspect of this optical imaging technique, also the potential role for uro-oncology diagnostics and the current state of development towards clinical implementation in the upper urinary tract, bladder, prostate, penis, kidney and testis are discussed.

In **Chapter 5**, the diagnostic ability of quantitative OCT analysis for UTUC grading is elaborated. The quantitative analysis relies on the exponential decay of the OCT signal with tissue depth (i.e. attenuation coefficient). The attenuation coefficients from in-vivo ureterorenoscopic OCT images of low-grade and high-grade UTUC are compared to evaluate the diagnostic ability for grade differentiation with the proposal of an attenuation coefficient cut-off value.

In **Chapter 6**, the use of CLE during endoscopic procedures for assessment of urothelial carcinoma is described. Furthermore, the study protocols of two prospective explorative studies, to assess the diagnostic ability of in-vivo CLE for diagnosis and grading of urothelial carcinoma in the bladder and the upper urinary tract, are presented.

Chapter 7 provides the results of the explorative study, in which the diagnostic ability of in-vivo CLE for UTUC is investigated. Furthermore, the earlier proposed CLE criteria for UTUC grading are validated and a refined CLE scoring system for grading of UTUC during ureterorenoscopy is presented.

In **Chapter 8**, a feasibility study is presented, in which the use of FISH is investigated for the detection of UTUC in selectively collected urine samples of 1 mL from the upper urinary tract. Implementation of urine-based detection methods, which can be performed in an outpatient setting, could lead to a reduction of the number of ureterorenoscopic examinations during the follow-up after kidney-sparing treatment, and hence a reduction of patient burden.

In **Chapter 9,** the thesis is finalized with a general discussion of the findings and a future outlook on the challenges and developments ahead in the diagnostics of UTUC.

The **appendix** provides an English, Dutch and German summary of the thesis.

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Chapter 2

The Diagnostic Yield and Concordance of Ureterorenoscopic Biopsies for Grading of Upper Tract Urothelial Carcinoma: A Dutch Nationwide Analysis

J.E. Freund, M.J.C. Duivenvoorden, B.T. Sikma, R.W.M. Vernooij, C.D. Savci-Heijink, J.D. Legemate, T.M. de Reijke
J Endourol. 2020;34(9):907-13.

List of abbreviations

CI – confidence interval
CIS – carcinoma in situ
IQR – interquartile range
NPV – negative predictive value
PALGA – Dutch nationwide network and registry of histo- and cytopathology
PPV – positive predictive value
PUNLMP – papillary urothelial neoplasms of low malignant potential
RU – renal unit
TNM – tumour node metastasis
UTUC – upper tract urothelial carcinoma
WHO – World Health Organization

Abstract

Objectives: To evaluate the diagnostic yield and concordance of upper tract urothelial carcinoma (UTUC) grading between ureterorenoscopic biopsies and surgical resections.

Materials and Methods: The nationwide Dutch Pathology Registry (nationwide network and registry of histo- and cytopathology in the Netherlands [PALGA]) was searched for UTUC-positive renal units (RUs) with histopathology excerpts from ureterorenoscopic biopsies and surgical resections, matched for laterality and localization of the tumour, from 2011 until 2018. The positive predictive value (concordance) of the biopsy grade with regard to the final grade according to the World Health Organization (WHO) 2004 classification was calculated.

Results: A total of 1002 UTUC-positive renal units were included, of which 776 UTUC-positive RUs were graded according to the WHO 2004 classification in the ureterorenoscopic biopsy, the localization-matched surgical resection, or in both. The diagnostic yield of biopsies for a classifying diagnosis was 89% with a sensitivity for UTUC of 84%. In case of UTUC, the diagnostic yield for biopsy-based grading and staging was 97% and 72%, respectively. The concordance of high-grade biopsies with regard to the final histopathology was 97% and 62% for low-grade biopsies. Upgrading to final high grade occurred in 33% of the low-grade biopsies. Downgrading to final low grade occurred in 2% of high-grade biopsies.

Conclusions: This is the first study to portray the limitations of ureterorenoscopic biopsies for UTUC in a nationwide cohort. The diagnostic yield of ureterorenoscopic biopsies for a classifying diagnosis is suboptimal, but the diagnostic yield for grading according to the WHO 2004 classification is high. Yet, a worrisome amount of ureterorenoscopic biopsies are upgraded with regard to the surgical resection. Consequently, one-third of patients, who qualify for kidney-sparing treatment according to one of the criteria recommended for risk stratification, might be stratified incorrectly. These findings stress the importance of a timely and stringent ureterorenoscopic follow-up after kidney-sparing surgery and highlight the need for improvements in the diagnostic approach to optimize the risk stratification.

Introduction

Upper tract urothelial cancer (UTUC) is a rare cancer with an annual incidence of 2 cases per 100,000 inhabitants in Western countries.[1–3] Based on the histologic architecture, UTUC can be divided into papillary tumours and flat lesions [carcinoma in situ (CIS)]. UTUC is treated by surgical resection (partial ureterectomy or radical nephroureterectomy) or in selected cases by ureterorenoscopic laser ablation.[1] Patient selection for ureterorenoscopic ablation is mainly based on the extent of the disease and the histopathologic grade.[1,4,5] As a result, ureterorenoscopic biopsies and their histopathologic assessment are essential to the diagnostic paradigm if radiologic imaging and cytology do not provide diagnostic certainty; especially if kidney-sparing treatment is considered.[1] The diagnostic yield and the diagnostic accuracy of ureterorenoscopic biopsies for grading and staging, however, are a point of discussion.[6–13] Despite the recommended risk-stratification, the risk of undergrading with ureterorenoscopic biopsies might lead to suboptimal oncologic outcomes with ureterorenoscopic ablation.

To our knowledge, nationwide cohort studies, evaluating the abovementioned issues, are lacking. This first nationwide study investigates the diagnostic yield and accuracy of ureterorenoscopic biopsies for UTUC grading. Based on a nationwide Dutch cohort from 2011 until 2018, the diagnosis and the histopathologic grade of ureterorenoscopic biopsies are compared with the findings of the corresponding surgical resection specimens. Additionally, the diagnostic accuracy of biopsy-based grading for the prediction of the definitive histopathologic stage is assessed.

Materials and Methods

Data collection

The nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) was used for data collection. The PALGA includes nationwide coverage of all academic and non-academic medical centers since 1991.[14] Standardized coding of diagnoses, including anatomical locations, and pseudonymization allow for anonymized population-based epidemiological studies. The PALGA was searched from 01-01-2011 until 01-01-2018 separately for all biopsies and all resections from the ‘ureter’, ‘renal pelvis’ or ‘kidney’ with ‘urothelial carcinoma’, ‘urothelial carcinoma in situ’ or ‘carcinoma in situ’. Duplicates were removed.

Histopathologic variables, alongside gender and patient age, were extracted. The histopathologic variables of the biopsy and surgical resection included date and type of procedure, laterality, tumour location, diagnosis, histopathologic stage (TNM 2002) and grade according to the WHO 2004 classification. Specific information on the endoscopic instruments used was not available.

UTUC-positive RU's with histopathology excerpts from ureterorenoscopic biopsies and successive ipsilateral surgical resections (partial ureterectomy, nephrectomy or nephroureterectomy) with matching tumour localization, were included ('Cohort 1' in Figure 1). RU's with >120 days between the biopsy and the successive resection were excluded. The diagnostic yield of biopsies was calculated with 'Cohort 1'. 'Cohort 1' was subdivided into 'Cohort 2004' to select UTUC-positive renal units with grading according to the WHO 2004 classification in either the ureterorenoscopic biopsies, the surgical resection or in both (Figure 1).

According to the abovementioned, the concordance of UTUC grading, based on the WHO 1973 classification was also assessed ('Cohort 1973' in Figure 1). The results of 'Cohort 1973' are presented in the supplementary data.

Figure 1; inclusion/exclusion flow chart

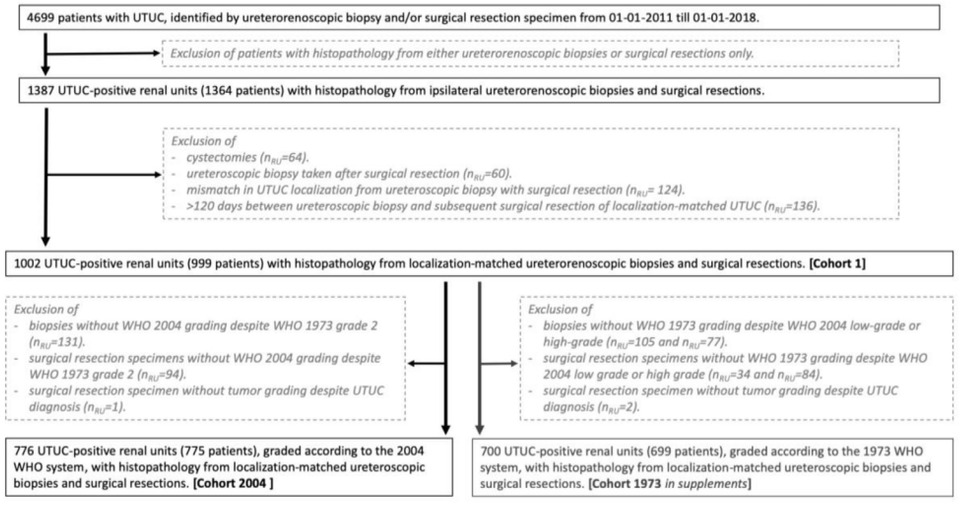

Figure legends: Figure 1 illustrates the inclusion and exclusion of identified cases for the general cohort (Cohort 1) and its subdivision according to the WHO grading classification used into Cohort 2004 and Cohort 1973 for final analysis. (The results of Cohort 1973 are listed in the supplementary data.)

Statistical analysis
The demographic and histopathologic variables were assessed with descriptive statistics. The diagnostic yield was defined as the percentage of conclusive biopsies and was calculated for diagnosis and grade, accordingly. The reference standard was the histopathology of the resection specimen.

The grade and stage from the ureterorenoscopic biopsies were referred to as the 'biopsy grade' and 'biopsy stage', and from the surgical resections as 'final grade' and 'final stage'.

Based on 2x2 tables, the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) of the biopsy grade with regard to the final grade were calculated for 'Cohort 2004'. Papillary urothelial neoplasms of low malignant potential (PUNLMP) were considered as low-grade UTUC.

Similarly, the sensitivity, specificity, NPV and PPV of the biopsy grade to predict the final stage were calculated with 2x2 tables for papillary UTUC of 'Cohort 2004'. The final stage was divided into two dichotomous groups (Ta versus ≥T1 & ≤T1 versus ≥T2) to assess the predictive value of the biopsy grade for final stage. The probability of lack of invasion (Ta) or lack of muscle invasion (≤T1) were calculated for low-grade biopsies. The probability of invasive growth (final ≥T1) and muscle invasion (final ≥T2) were calculated for high-grade biopsies.

Data analysis was performed with SPSS v.24.0 (IBM, Armonk, NY, USA).

Results

From 01-01-2011 until 01-01-2018, 4699 patients with UTUC-positive biopsies and/or resection specimens were identified. Of these patients, 999 patients had received ureterorenoscopic biopsies and successive resections in a total of 1002 RU's ('Cohort 1' in Figure 1). The 999 patients comprised 31% female and 69% male patients with a mean age at diagnosis of 67 years (±9 SD, range 33 – 93 years). In 'Cohort 1', 776 RU's with a UTUC-positive biopsy and resection were graded according to the WHO 2004 classification ('Cohort 2004').

Ureterorenoscopic biopsy
As illustrated in Table 1 for 'Cohort 1', the ureterorenoscopic biopsy resulted in insufficient material for a classifying diagnosis in 11% of the RU's. This means that the diagnostic yield of biopsies for a classifying diagnosis was 89%. UTUC was identified by biopsy in 824 of 1002 (82%) RU's. In case of UTUC, the diagnostic yield of ureterorenoscopic biopsies for UTUC grading was 97%

(799/824 UTUC-positive biopsies), and 72% for staging (597/824 UTUC-positive biopsies). An overview of the biopsy findings is shown in Table 1.

Surgical resection

The median time from ureterorenoscopic biopsy until surgical resection was 44 days (IQR 33-60). In the resection specimens, UTUC was diagnosed in 975 of the 1002 RU's, comprising 22 RU's with solitary CIS (2%), 835 RU's with papillary UTUC (86%), and 118 RU's with papillary UTUC and concomitant CIS (12%). Grade and stage were not reported in 2 of 975 UTUC-positive resections. An overview of findings by surgical resection is presented in Table 2.

Table 1: Findings of ureterorenoscopic biopsies for each cohort.

		'Cohort 1' frequency (%) *(n=1002 renal units)*	**'Cohort 2004'** frequency (%) *(n=776 renal units)*
Biopsy localization	Ureter	586 (59)	455 (59)
	Pyelocaliceal system	388 (39)	296 (38)
	Ureter and pyelocaliceal system	27 (3)	24 (3)
	Not reported	1 (0)	1 (0)
Diagnosis	Urothelial carcinoma	824 (82)	617 (80)
	Renal cell carcinoma	1 (0)	1 (0)
	Reactive tissue/no malignancy	72 (7)	65 (8)
	Insufficient material for diagnosis	105 (11)	93 (12)
UTUC grading	Any grading reported	799 (80)	
	No grading reported despite UTUC	25 (3)	
	No UTUC diagnosis	178 (18)	
Tumour grade (WHO 2004)	PUNLMP	11 (1)	11 (1)
	Low-grade	395 (39)	331 (43)
	High-grade	225 (23)	215 (28)
	Carcinoma in situ	37 (4)	37 (5)
	Not reported/insufficient material for grading	156 (16)	23 (3)
	No UTUC/insufficient material for diagnosis	178 (18)	159 (21)
Tumour grade (WHO 1973)	Grade 1	212 (21)	
	Grade 2	248 (25)	
	Grade 3	120 (12)	
	Carcinoma in situ	37 (4)	
	Not reported/insufficient material for grading	207 (21)	
	No UTUC/insufficient material for diagnosis	178 (18)	
Pathologic stage	Ta	455 (45)	327 (42)
	Tis	37 (4)	37 (5)
	T1	99 (10)	79 (10)
	≥T2	16 (2)	14 (2)
	Not reported/insufficient material for staging	217 (22)	160 (21)
	No UTUC/insufficient material for diagnosis	178 (18)	159 (20)
Median time in days between biopsy and surgical resection [IQR]		44 [33 - 59]	44 [34 - 59]

Table legends: IQR = interquartile range, PUNLMP = papillary urothelial neoplasm of low malignant potential, UTUC = upper tract urothelial carcinoma, WHO = World Health Organization.

Table 2: Findings of surgical resections for 'Cohort 1' and 'Cohort 2004'.

		'Cohort 1' frequency (%), *(n=1002 RU)*	**'Cohort 2004'** frequency (%), *(n=776 RU)*
Type of resection	Nephroureterectomy	646 (65)	504 (65)
	Nephrectomy	179 (18)	134 (17)
	Segmental ureter resection	177 (18)	138 (18)
Diagnosis	Urothelial carcinoma	975 (97)	756 (97)
	Renal cell carcinoma	4 (0)	3 (0)
	Reactive changes of urothelium	21 (2)	15 (2)
	Other diagnosis	2 (0)	2 (0)
Tumour localization	Ureter	549 (55)	430 (55)
	Pyelocaliceal system	390 (39)	301 (39)
	Ureter and pyelocaliceal system	36 (4)	27 (4)
	Other diagnosis than UTUC	27 (3)	18 (2)
UTUC grading	Any grading reported	973 (97)	
	No grading reported despite UTUC	2 (0)	
	No UTUC diagnosis	27(3)	
Tumour grade (WHO 2004)	PUNLMP	2 (0)	2 (0)
	Low-grade	287 (29)	263 (34)
	High-grade	525 (52)	470 (61)
	Solitary carcinoma in situ	22 (2)	21 (3)
	Not reported	139 (14)	0 (0)
	Other diagnosis than UTUC	27 (3)	20 (3)
Tumour grade (WHO 1973)	Grade 1	77 (8)	
	Grade 2	343 (34)	
	Grade 3	341 (34)	
	Solitary carcinoma in situ	22 (2)	
	Not reported	192 (19)	
	Other diagnosis than UTUC	27 (3)	
Presence of concurrent carcinoma in situ	Yes	118 (12)	102 (13)
	No	835 (83)	633 (82)
	Other diagnosis/solitary CIS	49 (5)	41 (5)
Pathologic stage	Ta	399 (40)	280 (36)
	Tis	22 (2)	21 (3)
	T1	144 (14)	109 (14)
	T2	144 (14)	119 (15)
	T3	231 (23)	199 (26)
	T4	19 (2)	16 (2)
	Not reported	16 (2)	12 (2)
	Other diagnosis than UTUC	27 (3)	20 (3)
Median tumour diameter in cm [IQR]		3.0 [1.8 - 4.0]	3.0 [1.9 - 4.2]

Table legends: CIS = carcinoma in situ, IQR = interquartile range, PUNLMP = papillary urothelial neoplasm of low malignant potential, RU = renal unit, UTUC = upper tract urothelial carcinoma, WHO = World Health Organization.

Concordance

In 814 of the 975 RU's with UTUC-positive resections, UTUC was diagnosed with ureterorenoscopic biopsies, resulting in a sensitivity of 84% for UTUC.

Biopsy-based low-grade and high-grade UTUC were reported in 43% and 28% of the 776 RU's with both UTUC-positive biopsies and resections, respectively (Table 1). In contrast, low-grade and high-grade UTUC were reported in 34% and 61% of the resection specimen (Table 2). In comparison with the surgical resection specimen, low-grade UTUC was correctly identified by biopsy in 213 of 265 RU's (80% sensitivity). Two-hundred-thirteen of 342 biopsies with low-grade UTUC were in concordance with the final grade (62% PPV). Upgrading to final high-grade UTUC occurred in 33% of biopsies (114/342). In 15 of 342 RU's

(4%) with a low-grade biopsy, UTUC was not anymore identified in the resection specimen.

High-grade UTUC was correctly identified by biopsy in 244 of 491 RU's (50% sensitivity). Two-hundred-forty-four of 252 biopsies with high-grade UTUC were in concordance with the final grade (97% PPV). Downgrading to final low-grade occurred in 2% of biopsies (4/252). UTUC was not identified anymore in the resection specimen in 2% of RU's with a high-grade biopsy. The specificity and NPV of low-grade and high-grade biopsies with regard to the final grade are presented in Table 3.

Table 3: Diagnostic accuracy of ureterorenoscopic biopsies for grading according to the WHO 2004 classification.

	Sensitivity (%) [95% CI]	***Specificity*** (%) [95% CI]	**Positive predictive value** (%) [95% CI]	***Negative predictive value*** (%) [95% CI]
Low-grade biopsy	80.4 [75.1 - 85.0]	74.8 [70.8 - 78.5]	62.3 [58.4 - 66.0]	88.0 [85.1 - 90.4]
High-grade biopsy	49.7 [45.2 - 54.2]	97.2 [94.5 - 98.8]	96.8 [93.9 - 98.4]	52.9 [50.6 - 55.1]

Table legends: CI = confidence interval

Stage prediction with biopsy grade

As shown in Table 4, a total of 210 of 301 RU's with final stage Ta were diagnosed as low-grade UTUC by biopsy (sensitivity 70%). The final stage was Ta in 210 of 342 biopsies with a low-grade biopsy (PPV 61%). For UTUC with final stage ≤T1, the sensitivity of biopsies with biopsy low-grade was 63% (258/410) with a PPV of 75% (258/342).

A total of 173 of 443 RU's with final stage ≥T1 were diagnosed as high-grade UTUC by biopsy (sensitivity of 39%). In 173 of 215 biopsies with high-grade UTUC, the final stage was ≥T1 (PPV 81%). For UTUC with final stage ≥T2, the sensitivity of biopsy high-grade UTUC was 42% (141/334) with a PPV of 66% (141/215).

Table 4: Diagnostic accuracy of the histopathologic grade (WHO 2004) from ureteroscopic biopsies for the prediction of the pathologic stage in the surgical resection specimen.

	Sensitivity (%) [95% CI]	***Specificity*** (%) [95% CI]	**Positive predictive value** (%) [95% CI]	***Negative predictive value*** (%) [95% CI]
Low-grade biopsy for prediction of Ta	69.8 [64.2 - 74.9]	72.2 [68.0 - 76.2]	61.4 [57.5 - 65.2]	79.0 [75.9 - 81.9]
Low-grade biopsy for prediction of ≤T1	62.9 [58.1 - 67.6]	77.0 [72.4 - 81.3]	75.4 [71.5 - 79.0]	65.0 [61.8 - 68.1]
High-grade biopsy for prediction of ≥T1	39.1 [34.5 - 43.8]	87.4 [83.3 - 90.8]	80.5 [75.2 - 84.8]	51.9 [49.8 - 54.0]
High-grade biopsy for prediction of ≥T2	42.2 [36.9 - 47.7]	83.3[79.4 - 86.6]	65.6 [59.9 - 70.8]	65.6 [63.3 - 67.8]

Table legends: CI = confidence interval, Ta = no invasive growth (TNM 2002), T1 = invasion of the lamina propria (TNM 2002), T2 = muscle invasion (TNM 2002).

Discussion

Ureterorenoscopic biopsies and their histopathologic assessment are essential to the diagnostic paradigm if radiology and cytology do not provide diagnostic certainty; especially if kidney-sparing treatment is considered.[1,5,15] The diagnostic yield and the diagnostic accuracy of ureterorenoscopic biopsies, however, may be limited due to anatomical and technical limitations.[5,7,16] Despite recommendations for careful risk-stratification, the diagnostic limitations and the risk of undergrading pose a risk for the oncologic outcome of kidney sparing surgery.[5–7]

Previous studies, which investigated the accuracy of UTUC grading, are of limited sample size. The limited numbers can be explained by the low prevalence of UTUC. For this reason, the clustering of data is of paramount importance for evaluations of disease-specific outcomes. We present the first nationwide and largest retrospective study to assess the diagnostic yield and concordance of ureterorenoscopic biopsies for UTUC.

In our study, ureterorenoscopic biopsies show a diagnostic yield of 89% for a classifying diagnosis with a sensitivity of 84% for UTUC. In case of UTUC-positive biopsies, the diagnostic yield for UTUC grading is high (97%). Yet, a high rate of upgrading occurs with regard to the surgical resection (33% of low-grade UTUC). Moreover, the diagnostic yield of ureterorenoscopic biopsies for UTUC staging is limited (72 %), while biopsy-based high-grade papillary UTUC holds an acceptable PPV (81%) to predict invasion of the lamina propria or worse (≥T1). It is likely that the abovementioned findings result from sampling error (insufficient material, crush artefacts) and diagnostic error. Tumour specific factors, such as grade heterogeneity or disease progression, might also contribute to the low concordance.

The identified diagnostic yield for a classifying diagnosis is in line with the study by Wang et al.[10] In a smaller study by Tavora et al, 25% of ureterorenoscopic biopsies lead to a non-diagnostic result owing to sampling error.[13] Similarly, our diagnostic yield of ureterorenoscopic biopsies for UTUC grading is at the higher end of the range that is described in the literature, varying from 65% to 100%.[6–13,17] The difference with the lowest reported diagnostic yield may be explained by their inclusion of cases from before the era of modern ureterorenoscopy.[9]

The concordance of biopsy high-grade UTUC with regard to the final histopathology is high (PPV 97%). The low concordance of biopsy-based low-grade UTUC with the final histopathology (PPV 62%) is, however, worrisome. Smith et

al and Margolin et al reported a similar rate of upgrading for low-grade biopsies (43% and 51%, respectively), while two smaller studies reported a concordance of 87% and 93%.[6,7,17,18] As one third of low-grade biopsies are upgraded with regard to the final histopathology, one third of patients, who qualify for kidney-sparing treatment according to one of the criteria recommended for risk-stratification, might be stratified incorrectly. Consequently, these findings stretch the importance of a timely and stringent ureterorenoscopic follow-up after kidney sparing surgery to safeguard the oncologic outcome.[19]

With regard to the WHO 1973 classification, we identified even higher rates of upgrading (69% of G1 biopsies and 34% of G2 biopsies). Also, Wang et al identified high rates of upgrading with the WHO 1973 classification.[10] Yet, in contrast to the WHO 2004 classification, the clinical implication of the rate of upgrading with the WHO 1973 classification is less readily assessible because the WHO 1973 classification is not incorporated in the recommended risk-stratification.[1] Subsequently, the WHO 1973 classification is outdated with regard to the current guidelines. Grading should be performed according to the WHO 2004/2016 classification.

Next, our study confirms the limited diagnostic yield of ureterorenoscopic biopsies for biopsy staging (72%).[6,7] Due to the limited diagnostic yield, the concordance of biopsy stage with final stage was not assessed in our study. The biopsy grade can be a tool to predict the final stage.[7] In line with Margolin et al, we identified an acceptable PPV (81%) for biopsy high-grade UTUC to predict invasive growth in the resection specimen (≥T1). The PPV of biopsy high-grade UTUC to predict final muscle invasion (≥T2), however, was lower (66%). This finding is notable as solitary CIS had already been considered a false negative or false positive because solitary CIS would have led to an underestimation of final muscle invasion for high-grade. Undergrading of high-grade UTUC with ureterorenoscopic biopsies may explain the observed difference in PPV. The PPV of 66% for biopsy high-grade to predict ≥T2 in the final histopathology is, nevertheless, in line with the current literature.[7,9] For biopsy low-grade lesions to predict the lack of muscle invasive (≤T1), the PPV was 75%. This finding suggests that despite low tumour grade, a substantial number of cases might show muscle invasion while being stratified for laser ablative treatment. The extent and the impact on the clinical outcome remain to be defined.

The present study is the first nationwide cohort to address the diagnostic yield and concordance of UTUC. The strengths of this study arise from the large sample size and its nationwide character. This study was only feasible owing to the nationwide, pathology-initiated PALGA registry. Even though collective data collection is of paramount importance for studies of less prevalent aetiologies,

nationwide databases are still rare. On the downside, retrospective analyses impose several limitations.

The lack of clinical follow-up data hinders the analysis of the clinical impact of upgrading. Similarly, the number of nephroureterectomies might be underreported. If the excerpts of surgically removed kidneys did not mention terms that indicated a nephroureterectomy or bladder cuff resection, the surgical resection was considered a nephrectomy. This may explain the high number of nephrectomies reported in this study.

The type of ureterorenoscopy and biopsy technique were also not reported in the PALGA database. This lack of information may introduce bias. Restricting the study period to 2011 until 2018 reduces at least some heterogeneity of data that may arise from technical aspects from the pre-modern ureterorenoscopy era. However, as reported in the literature, further assessment of the impact of biopsy volume on the diagnostic accuracy would have been desirable. [18,20,21]

Furthermore, as the PALGA registry only records events with histopathologic assessments, it was unknown if cases had undergone intermediate laser ablative treatment prior to surgical resection. To limit the potential number of cases with intermediate laser ablation, cases with more than 120 days between the diagnostic ureterorenoscopy and the surgical resection were excluded. This cut-off was set with regard to the recommendations of the European Urology guidelines to perform a resection within three months following diagnosis.[1]

The comparison of the histopathology from ureterorenoscopic biopsies and surgical resections introduces selection bias as a smaller fraction of low-grade UTUC is treated by surgical resection than high-grade UTUC. The reported 3 cm mean tumour diameter illustrates similar selection bias with this methodology towards potentially more aggressive tumour biology than would be the case for endoscopic management cohorts. The implication of these confounders is difficult to define and inevitable for retrospective diagnostic studies.

Lastly, the nationwide character of the study introduces interobserver variability of histopathologic assessment. However, the study still reflects the current clinical practice in the Netherlands. Reviewing the histopathology was not feasible in the present study setting, but could have been used to reduce bias.

Furthermore, improved collaborations and protocolization of clinical inquiries and histopathologic reports would benefit the quality of data in the PALGA registry greatly. The centralization of care, especially for rare diseases, would

also reduce the heterogeneity of data. Centralization of care might also lead to an improved diagnostic yield and concordance of ureterorenoscopic biopsies for UTUC, and hence improved oncologic outcomes. However, protocolization and centralization will not overcome the limitations of the current diagnostic approach and the potential pitfall of the risk-stratification for UTUC. The true challenge in this field is to find new ways to distinguish between low-grade and high-grade UTUC objectively and accurately.

Emerging optical imaging modalities such as confocal laser endomicroscopy and optical coherence tomography may hold the potential to augment the diagnostic accuracy of grading and staging.[22–25] Only with improvements in the diagnostic paradigm, we can deliver timely and curative single modality treatments without over-treatment or under-treatment to some, while others are directed to safe renal-sparing ureterorenoscopic treatment with ureterorenoscopic follow-up, and select multimodality therapy for a third subset who may have more aggressive features beyond histopathologic grading and staging.

Conclusions

This is the first study to portray the limitations of ureterorenoscopic biopsies for grading and staging of UTUC in a nationwide cohort. The diagnostic yield of ureterorenoscopic biopsies for a classifying diagnosis is suboptimal. In case of UTUC, the diagnostic yield for histopathologic grading according to the WHO 2004 classification is nevertheless high. Yet, a worrisome amount of ureterorenoscopic biopsies are upgraded with regard to the surgical resection. Consequently, one third of patients, who qualify for kidney-sparing treatment according to one of the criteria recommended for risk-stratification, might be stratified incorrectly. Thereupon, these findings stress the importance of a timely and stringent ureterorenoscopic follow-up after kidney sparing surgery to safeguard the oncologic outcome. The results also highlight the need for improvements in the diagnostic approach to optimize individual treatment selection.

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Supplementary Data

Concordance of WHO 1973 grading

'Cohort 1973' consists of 700 RU's with a histopathologic diagnosis of UTUC, graded according to the WHO 1973 classification, from either a ureterorenoscopic biopsy, surgical resection or both. The histopathologic findings of the 'Cohort 1973' for the ureterorenoscopic biopsies are shown in Table S1 and for the surgical resections in Table S2.

In comparison with the histopathologic findings of the surgical resections, G1 was identified correctly by biopsy in 50 of 65 RU's (sensitivity 77%). In total, 50 of 187 G1 biopsies were in concordance with the final grade (PPV 27%). In total, upgrading of G1 biopsies occurred in 69% of the RU's (129/187). Upgrading to final G2 occurred in 58% of G1 biopsies (109/187); while upgrading to G3/CIS occurred in 11% of G1 biopsies (20/187). UTUC was not identified anymore in the resection specimen of 4% of G1 biopsies (8/187).

G2 was correctly identified by biopsy in 125 of 303 RU's (sensitivity 41%). In total, 125 of 212 G2 biopsies were in concordance with the final grade (PPV 59%). Upgrading to final G3/CIS occurred in 34% of G2 biopsies (72/212); while downgrading to final G1 occurred in 2% (4/212). UTUC was not anymore identified in the resection specimen of 5% of G2 biopsies (11/212).

In comparison with the surgical resection specimen, G3 was correctly identified by biopsy in 123 of 309 RU's (sensitivity 39%). In total, 123 of 137 G3 biopsies were in concordance with the final grade (PPV 90%). Downgrading to final G2 occurred in 8% of G3 biopsies (11/137). There was no downgrading of G3 biopsies to final G1. UTUC was not identified anymore in the resection specimen of 2% of G3 biopsies (3/137).

Additionally, the specificity and NPV of G1, G2 and G3 biopsies are presented in Table S3.

Table S1: Findings of ureterorenoscopic biopsies for 'Cohort 1973'.

		'Cohort 1973' frequency (%) *n=700 RU*
Biopsy localization	Ureter	412 (59)
	Pyelocaliceal system	265 (38)
	Ureter and pyelocaliceal system	22 (3)
	Not reported	1 (0)
Diagnosis	Urothelial carcinoma	559 (80)
	Renal cell carcinoma	0 (0)
	Reactive tissue/no malignancy	59 (8)
	Insufficient material for diagnosis	82 (12)
Tumour grade (WHO 1973)	Grade 1	187 (27)
	Grade 2	212 (30)
	Grade 3	106 (15)
	Carcinoma in situ	31 (4)
	Not reported/insufficient material for grading	23 (3)
	No UTUC/insufficient material for diagnosis	141 (20)
Pathologic stage	Ta	322 (46)
	Tis	31 (4)
	T1	71 (10)
	≥T2	11 (2)
	Not reported/insufficient material for staging	114 (18)
	No UTUC/insufficient material for diagnosis	141 (20)
Median time in days between biopsy and surgical resection [IQR]		44 [33 - 60]

Table legends: IQR = interquartile range, PUNLMP = papillary urothelial neoplasm of low malignant potential, RU = renal unit, UTUC = upper tract urothelial carcinoma, WHO = World Health Organization.

Table S2: Findings of surgical resections for 'Cohort 1973'.

		'Cohort 1973' frequency (%) *n=700 RU*
Type of resection	Nephroureterectomy	439 (63)
	Nephrectomy	128 (18)
	Segmental ureter resection	133 (19)
Diagnosis	Urothelial carcinoma	677 (97)
	Renal cell carcinoma	3 (0)
	Reactive changes of urothelium	18 (3)
	Other diagnosis	2 (0)
Tumour localization	Ureter	385 (55)
	Pyelocaliceal system	269 (38)
	Ureter and pyelocaliceal system	23 (3)
	Other diagnosis than UTUC	23 (3)
Tumour grade (WHO 1973)	Grade 1	65 (9)
	Grade 2	303 (43)
	Grade 3	290 (41)
	Solitary carcinoma in situ	19 (3)
	Not reported	0 (0)
	Other diagnosis than UTUC	23 (3)
Presence of concurrent carcinoma in situ	Yes	83 (12)
	No	575 (82)
	Other diagnosis/solitary CIS	42 (6)
Pathologic stage	Ta	288 (41)
	Tis	19 (3)
	T1	101 (14)
	T2	94 (13)
	T3	150 (21)
	T4	13 (2)
	Not reported	12 (2)
	Other diagnosis than UTUC	23 (3)
Median tumour diameter in cm [IQR]		2.7 [1.5 - 4.0]

Table legends: CIS = carcinoma in situ, IQR = interquartile range, PUNLMP = papillary urothelial neoplasm of low malignant potential, RU = renal unit, UTUC = upper tract urothelial carcinoma, WHO = World Health Organization.

Table S3: Diagnostic accuracy of ureterorenoscopic biopsies for grading according to the WHO 1973 classification.

	Sensitivity (%) [95% CI]	***Specificity*** (%) [95% CI]	**Positive predictive value** (%) [95% CI]	***Negative predictive value*** (%) [95% CI]
G1 biopsy	76.9 [64.8 - 86.5]	78.4 [75.0 - 81.6]	26.7 [23.0 - 30.8]	97.1 [95.5 - 98.1]
G2 biopsy	41.3 [35.7 - 47.0]	78.1 [73.7 - 82.1]	59.0 [53.3 - 64.4]	63.5 [61.0 - 64.4]
G3 biopsy	39.8 [34.3 - 45.5]	96.4 [94.1 - 98.0]	89.8 [83.8 - 93.7]	67.0 [64.9 - 69.0]

Table legends: CI = confidence interval.

Chapter 3

Upper Tract Urothelial Carcinoma Grade Prediction based on the ureteroscopic Appearance: Caution should be taken

J.E. Freund, J.D. Legemate, J. Baard, K. Saeb-Parsy, O. Wiseman, S. Doizi, E. Emiliani, A. Breda, B.J. Boodt, E.P. van Haarst, M.M.G. Leeflang, G.M. Kamphuis

Urology. 2019;132:69-74.

List of abbreviations

CI – confidence interval
IQR – interquartile range
SD – standard deviation
UC – urothelial carcinoma
UTUC – upper tract urothelial carcinoma
WHO – World Health Organization

Abstract

Introduction: The histopathologic grade of upper tract urothelial carcinoma (UTUC) is essential to the risk-stratification for treatment selection. To highlight the current limitations of ureteroscopy, this study investigates the diagnostic accuracy, inter-rater and intra-rater agreement of histopathologic grade predictions based on the visual appearance of UTUC with digital ureteroscopy.

Methods: Nine urologists predicted the histopathologic grade of 64 UTUC (low-grade versus high-grade) by assessing the visual appearance of the tumours in videos from digital ureteroscopy. The diagnostic accuracy was estimated by comparing the grade predictions with the histopathology from co-localized biopsies. Inter-rater agreement was assessed by pairwise inter-rater percentage agreement and Fleiss Kappa analysis. Thirty days after prediction, the videos were rated again in a random order by the urologists to evaluate the intra-rater percentage agreement.

Results: Low-grade tumours were predicted correctly in 37% to 85% of the cases with a median concordance of 59% for questionnaire 1 and 66% for questionnaire 2. High-grade tumours were predicted correctly in 26% to 91% of the cases with a median concordance of 52% and 61% for each questionnaire. The median pairwise inter-rater percentage agreement was 66% for both questionnaires with a Fleiss Kappa of 0.29 and 0.38, respectively. The median intra-rater percentage agreement was 81%.

Conclusions: Predictions of the histopathologic grade of UTUC based on the visual appearance with digital ureteroscopy are often incorrect in comparison with biopsy results and yield low inter-rater agreement. Urologists must be aware of these limitations in the assessment of UTUC to warrant good clinical practice.

Introduction

Histopathologic assessment of urothelial carcinomas enables the subdivision into low-grade and high-grade tumours.[1] The histopathologic grade is a decisive factor for the risk-stratification of upper tract urothelial carcinoma (UTUC), stratifying the disease into low-risk and high-risk groups.[2] Adequate risk-stratification is necessary for treatment selection as ureteroscopic laser ablation is generally reserved for low-risk tumours and surgical resection is indicated for high-risk tumours.[3]

At present, the diagnostic pathway is generally based on radiologic imaging, cytology and most importantly diagnostic ureteroscopy with ureteroscopic biopsies of the suspicious tumours. The diagnostic yield of ureteroscopic biopsies, however, is limited.[4,5] It was reported that 10-15% of ureteroscopic biopsies are inconclusive.[4,5] This may lead to suboptimal treatment selection or to second diagnostic ureteroscopy.

In situations without histopathologic certainty, endourologists might be tempted to predict the tumour grade based on the ureteroscopic appearance of UTUC. Clinical decision making based on ureteroscopic grade predictions is not advocated in any clinical guidelines. Studies on the diagnostic limitations of grade predictions based on the visual appearance of UTUC with digital ureteroscopy, however, are lacking. Therefore, the objective of the present study is to investigate the diagnostic accuracy as well as the intra- and inter-rater agreement of grade predictions based on the visual appearance of UTUC with digital ureteroscopy. These outcomes help us understand the limitations in the diagnostic pathway for UTUC and raise critical awareness to warrant good clinical practice.

Methods

Study execution

Nine urologists from The Netherlands, Spain, France and the United Kingdom predicted the tumour grade based on the visual appearance of biopsy-proven UTUC in 64 videos from digital ureteroscopy. The videos were embedded in a questionnaire on an encrypted web-based platform (Data Management System, T&S Innovations). Thirty days after completing the questionnaire, the urologists were invited for a second questionnaire with the same videos in a random order.

The questionnaires' interface is illustrated in Figure 1. The tumour location of the depicted tumour was given to aid the orientation of the raters. All raters were informed that the depicted tumours were histopathologic confirmed urothelial carcinomas. The raters were blinded to tumour grade and any other clinical information. Each video sequence could be viewed unlimitedly by each rater. After visual assessment, the raters predicted the tumour grade in a dichotomous fashion (low-grade or high-grade). The raters were asked to score the video quality concerning grade assessability on a three-point Likert scale (low = 1, moderate = 2, high = 3) for each video before advancing to the next video. Additionally, the urologists reported their experience with cystoscopy and ureteroscopy of urothelial carcinomas.

The local institutional review board granted a waiver for this study as no additional activities in human subjects were involved in this study. Opt-out informed consent was obtained from all patients.

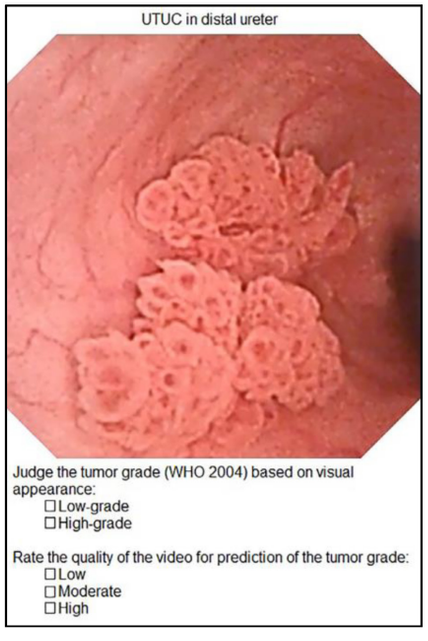

Figure 1: Example of the online questionnaire interface with the presentation of the video for upper tract urothelial carcinoma grade assessment.

Power calculation

The sample size calculation for the agreement analysis was based on an estimated intra- and inter-rater percentage agreement of 80% for grade predictions. The results of El-Hakim et al, who investigated the diagnostic accuracy of grade predictions based on pre-operative clinical information and the visual appearance of UTUC during fiber optic ureteroscopies, were used to estimate the agreement.[6] The power calculation resulted in an estimated sample size of 62 videos with 80% power and 10% alpha.

Video acquisition

The 64 videos were collected retrospectively from a video-database of the Academic Medical Center Amsterdam. This video-database was established for internal reviewing. The selected videos were acquired from 62 consecutive ureteroscopic procedures in 44 patients where a co-localized ureteroscopic biopsy of the depicted tumour confirmed urothelial carcinoma and the histopathologic grade.

All ureteroscopic procedures were performed with digital flexible ureteroscopes (52 procedures with Karl Storz Flex XC, 12 procedures with Olympus V2) in white light mode. Recording of the videos was performed with the Medicapture medical USB300 system and the Karl Storz Endoscope TC200 system. For all 64 cases, a high definition (HD) video sequence with a duration of 6 to 10 seconds was created with Apple iMovie v.9.0.9. The video sequences were chosen as such that the biopsied tumours were most optimally visualized from a distance and in detail.

Histopathologic reference standard

The histopathologic grade (WHO 2004) of co-localized biopsies from the depicted tumours was used as the reference standard.[1] The ureteroscopic biopsies were acquired with 1.1mm 3F Flexible Ureteroscopic Piranha Biopsy Forceps or a ZeroTipTM 0.63mm Nitinol Stone Retrieval Basket (both Boston Scientific). Histopathologic assessment was performed according to the standard clinical protocol by a uropathologist. The cohort consisted of 41 (64%) low-grade and 23 (36%) high-grade tumours. The included tumours were located in 28% (18/64) and in 72% (46/64) of the cases in the ureter or in the pyelocaliceal system, respectively.

Study outcomes and data analysis

Diagnostic accuracy calculations

The diagnostic accuracy was assessed in three ways:

1. the overall concordance of grade predictions with histopathology was calculated for each rater per questionnaire as proportions of percentage agreement, referred to as overall accuracy. Spearman's rank correlation testing was performed for the overall accuracy of grade predictions and the experience with urothelial carcinoma assessment of each rater.
2. the percentage of correctly predicted low-grade tumours of all biopsy-proven

low-grade tumours was calculated for each rater.
3. the percentage of correctly predicted high-grade tumours of all biopsy proven high-grade tumours was calculated for each rater.

Intra-rater agreement calculations

The intra-rater agreement was assessed by the percentage of cases where the raters predicted the same grade in both questionnaires, referred to as intra-rater percentage agreement. A threshold of minimally 80% agreement was considered as acceptable agreement.[7]

Inter-rater agreement calculations

The inter-rater agreement was determined in two ways:

1. for all pairwise combinations of the nine raters, the percentage of cases with the same grade prediction was determined. This is called the pairwise inter-rater percentage agreement. A threshold of minimally 80% agreement was considered as acceptable agreement.[7]
2. the overall Fleiss Kappa was calculated to correct for chance agreement, where a kappa statistic of <0.40 was defined as poor agreement.[8]

Descriptive statistics were used to illustrate tumour characteristics, rater characteristics, and video quality ratings. The Spearman's rank correlation coefficient was calculated for the total experience with urothelial carcinoma assessment and the overall accuracy of grade predictions. Statistical analyses were performed using SPSS Statistics v.24 and Matlab R2017b.

Results

Experience of the raters

The experience of the nine urologists with ureteroscopic UTUC assessment ranged from three to 14 years (mean 8.3 years) with a mean of 38 ureteroscopies for UTUC per year (SD 25.5). For cystoscopic assessment of urothelial carcinoma of the bladder, their experience ranged from six to 21 years (mean 11.9 years) with a mean of 142 cystoscopies per year (SD 96.7). (For the total number of endoscopic assessments of UC by each rater see Table S1 in the supplementary data.) There was no correlation between the total experience with urothelial carcinoma assessment and overall accuracy of grade predictions (Spearman's rank correlation coefficient = 0.23, p = 0.56).

Diagnostic accuracy

The overall accuracy of grade predictions and the percentages of correctly predicted low-grade and high-grade tumours are illustrated in Figures 2A–C. The median overall accuracy was 59% (IQR 12%) for questionnaire 1 and 64% (IQR 9%) for questionnaire 2. The median percentage of correctly predicted low-grade tumours was 59% (IQR 26%) for questionnaire 1 and 66% (IQR 26%) for questionnaire 2. For high-grade tumours, the median percentage of correct predictions was 52% (IQR 26%) for questionnaire 1 and 61% (IQR 26%) for questionnaire 2.

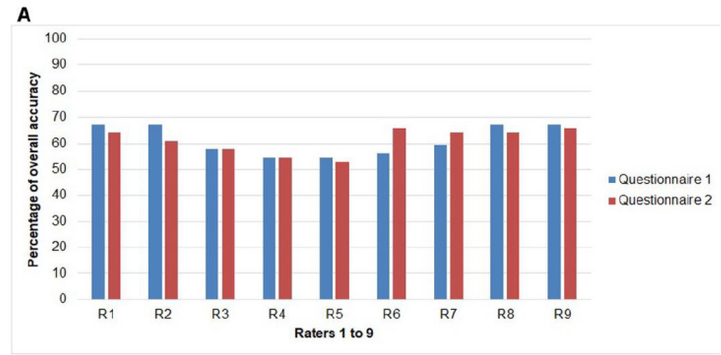

Figure 2: (A) Overall accuracy of grade predictions. (B) Percentage of correctly predicted low-grade tumours. (C) Percentage of correctly predicted high-grade tumours.

Intra-rater agreement
The intra-rater percentage agreement for the nine raters ranged from 55% to 91% with a median of 81% (IQR 13%). This median value is considered as an acceptable intra-rater percentage agreement.[7]

Inter-rater agreement
The pairwise inter-rater percentage agreement for questionnaire 1 ranged from 48% to 83% with a median of 66% (IQR 18%) (see Table S2 in supplementary data). This median value is below the threshold of acceptable percentage agreement.[7]

The Fleiss Kappa was 0.29 (95%CI: 0.28–0.30) for questionnaire 1 and 0.38 (95%CI: 0.37–0.39) for questionnaire 2. Hence, the overall inter-rater agreement was poor for both questionnaires.[8]

Video quality ratings
Based on the three-point Likert scale ratings, the overall mean video quality was 2.2 (SD 0.70) for questionnaire 1, and 2.3 (SD 0.68) for questionnaire 2. The overall mean video quality for cases with a correct grade prediction was 2.3 (SD 0.78). For cases with an incorrect grade prediction, the overall mean video quality was 2.2 (SD 0.76).

Discussion

The histopathologic tumour grade is a decisive factor for the risk-stratification of UTUC. As cytology and radiologic imaging are suboptimal diagnostic tools, ureteroscopy has become an essential tool to increase the diagnostic capabilities for UTUC.[2] Nevertheless, histopathologic grading of ureteroscopic biopsies is not always conclusive. Moreover, biopsies may not always be taken. In light of the results of this study, grade predictions based solely on the visual appearance of UTUC with digital ureteroscopy are too limited to provide diagnostic certainty. Grade predictions were incorrect in one third to half of all cases with a poor inter-rater agreement but acceptable intra-rater agreement.

This is the first study to investigate the diagnostic accuracy of grade predictions solely based on the visual appearance of UTUC in ureteroscopic images. A comparison of the diagnostic accuracy of grade predictions can be performed with the study by El-Hakim et al, which, however, had a different study design. In their study, operating room reports were checked retrospectively to investigate the diagnostic accuracy of grade predictions based on pre-operative clinical findings together with the visual appearance of UTUC during fiberoptic ureterorenoscopies. The extent and variation in additional clinical information available for grade predictions and potential inter-rater variability were not reported.[6] Forty cases were included in their final analysis, resulting in an overall accuracy of 70%. The median overall accuracy of our study was slightly lower for both questionnaires (59% and 64%).[6] El-Hakim et al reported a percentage of correctly predicted high-grade tumours of 47%, while we found a median percentage of correct predictions of 52% and 61% for the individual questionnaires. In contrast, the percentage of correctly predicted low-grade tumours by El-Hakim was 87%, while we identified a median percentage of 59% and 66% for the individual questionnaires. It is remarkable that the percentage of correctly predicted high-grade tumours by El-Hakim is lower despite the availability of additional clinical information. On the other hand, the value of additional clinical information may seem of additional value when looking at the difference in correctly predicted low-grade tumours. However, with the limited information at hand, one cannot draw conclusions about the origin of these differences.

Variations in the diagnostic accuracy of tumour grade predictions during cystoscopy of urothelial carcinoma of the bladder (UCB) have also been reported. Based on the visual appearance of UCB alone, Liem et al. correctly predicted the grade in 54% of low-grade UCB and in 67% of high-grade UCB.[9] In contrast, Cina et al correctly identified 91% of all low-grade UCB and Herr et al. correctly identified 93% of all grade 1 UCB.[10,11] Yet in the latter studies, additional clinical information was available. Furthermore, the absence of intermediate grade 2 tumours in the study by Herr et al. may have led to more distinctive visual differences between low-grade and high-grade tumours.[11]

In our study, the inter-rater agreement was poor and hence highlighting the limitations of grade predictions based on the ureteroscopic appearance of the tumour.[7,8] In contrast, Herr et al. observed an excellent agreement between raters for grade predictions of UCB.[11] This difference in inter-rater agreement may also be explained by the availability of additional clinical information in their study. One may also argue that the difference in inter-rater agreement could arise from differences in personal experience of the raters. In our study, however, the urologists' experience with assessment of urothelial carcinoma was not correlated with the overall accuracy of UTUC grade predictions.

The quality of the videos was assessed to evaluate if the video quality was a confounder for grade predictions. Overall, the video quality concerning the assessability of the tumour grade was rated as moderate with a tendency towards high quality. The video quality of the correctly predicted tumours was very similar to the image quality of the falsely predicted tumours. Therefore, it seems that the video quality did not limit nor influence grade prediction.

The outcome of this study shows that the visual appearance of UTUC during digital ureteroscopy alone is insufficient for accurate grade prediction. As a result, it seems disputable whether the histopathologic grade is distinctly characterized by the phenotypes of low-grade and high-grade UTUC. Nonetheless, future studies should investigate if widely available image enhancement techniques, such as Narrow Band Imaging or Image1S, may aid the assessment of the visual appearance. Moreover, convolutional neural networks and other image recognition software may be able to identify image patterns of UTUC that allow for accurate grade prediction with digital ureteroscopy.

The use of ureteroscopic biopsies as the reference standard is a point of discussion as biopsies may underestimate the histopathologic grade in comparison to resection specimens.[4,12] Therefore, one may argue that histopathology from surgical specimen is a superior reference standard. Yet, resection specimens may introduce selection bias due to treatment selection based on the histopathologic grade. Moreover, with regard to tumour grade heterogeneity between spatially separated unilateral tumours, co-localized biopsies may be more suitable as a reference standard for the assessment of focal regions of interest than histopathology from representative sampling of resection specimens.[13,14] In any case, the limitations of the reference standards may confine the accuracy of comparison.

To achieve the aim of this study, the raters were blinded to additional clinical information. Therefore, the findings of this study can be considered as the baseline diagnostic accuracy for grade predictions based on the visual appearance of UTUC. The additive diagnostic value of additional clinical information for grade predictions remains to be determined.

Despite careful selection of the video sequences for optimal visualization of the tumours in close-up and from a distance with the unlimited play-back time for each rater, the restriction in video length may have limited the visualization of the tumours. More extensive videos from more angles and visualization of the complete upper tract might aid grade prediction.

Conclusions

The histologic grade of UTUC is a decisive factor for the risk-stratification of the disease. The visual appearance of UTUC with digital ureteroscopes, however, does not allow for accurate predictions of the histopathologic grade. The diagnostic accuracy of grade predictions is limited and of low inter-rater agreement. Urologists must be aware of these limitations in the ureteroscopic assessment of UTUC to warrant good clinical practice.

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Supplementary Data

Table S1: The total number of ureteroscopic and cystoscopic assessments of urothelial carcinoma by each rater.

Rater	Total number of ureteroscopic assessments of UTUC performed	Total number of cystoscopic assessments of UC performed
R1	300	1800
R2	700	1200
R3	180	2100
R4	60	700
R5	450	1500
R6	420	510
R7	280	900
R8	60	450
R9	240	4200

Table S2: Pairwise inter-rater percentage agreement for questionnaire 1.

	Rater 1	Rater 2	Rater 3	Rater 4	Rater 5	Rater 6	Rater 7	Rater 8
Rater 2	69							
Rater 3	81	66						
Rater 4	78	69	78					
Rater 5	56	53	66	59				
Rater 6	55	73	52	61	48			
Rater 7	77	73	73	83	55	69		
Rater 8	66	66	50	53	56	48	64	
Rater 9	78	69	72	69	59	48	70	69

Chapter 4

Optical Coherence Tomography in Urologic Oncology: A Comprehensive Review

J.E. Freund, M. Buijs, C.D. Savci- Heijink, D.M. de Bruin, J.J.M.C.H. de la Rosette, T.G. van Leeuwen, M.P. Laguna
SN Compr Clin Med. 2019;1:67-84.

List of abbreviations

A-scan – *amplitude scan*
BC – *bladder cancer*
B-scan – *brightness scan*
CIS – *carcinoma in situ*
LoE – *level of evidence*
LP – *lamina propria*
NPV – *negative predictive value*
MP – *muscularis propria*
μ_{OCT} – *optical coherence tomography attenuation coefficient*
OCT – *optical coherence tomography*
PC – *prostate cancer*
PeC – *penile cancer*
RCC – *renal cell carcinoma*
RMB – *renal mass biopsy*
SCC – *squamous cell carcinoma*
SRM – *small renal mass*
UTUC – *upper tract urothelial carcinoma*
WHO – *World Health Organization*

Abstract

Introduction: Optical coherence tomography (OCT) is being investigated in urologic oncology for optical diagnosis. This comprehensive review analyses the current state of development of OCT for bladder, upper urinary tract, kidney, prostate, testis and penis cancer. Also, the potential role of OCT with regard to the current diagnostic pathways is critically appraised to guide future developments.

Methods: Embase and PubMed were systematically searched for English and German articles on OCT in humans up to December 2017. Reviews were excluded. Case reports were also excluded, unless they presented a landmark in the development of OCT.

Results: Out of 878 articles, 17 relevant articles on bladder, seven on kidney, five on upper urinary tract, four on prostate, and two on penile cancer were included. In these organs, in-vivo OCT imaging is feasible with potential for qualitative and quantitative diagnosis, grading and staging in specific organs. The development of OCT has reached IDEAL exploration stage 3 with 2b level of evidence. Relevant articles on testis cancer were lacking.

Conclusions: OCT allows for non- or minimally-invasive cancer diagnosis in the bladder, upper urinary tract, kidney, prostate and penis. In some organs OCT also may enable histologic grade and stage prediction. However, the current evidence is still at an exploratory level. With regard to the potential additional value of OCT in comparison to the current diagnostic pathways, OCT could become a diagnostic replacement or add-on test for urothelial carcinoma, penile carcinoma, and renal masses. Further research in these conditions should be encouraged.

Introduction

In quest of optimizing diagnostic pathways, new imaging modalities are constantly developed. Among them, optical imaging is riding on the crest of a wave with a good example on the rising interest in Optical Coherence Tomography (OCT).

OCT, also called the optical analogue of ultrasound imaging, facilitates in vivo high-resolution cross-sectional imaging of tissues. In contrast to ultrasound imaging, which uses time of flight measurements for depth ranging, OCT depth information is obtained by low coherence interferometry, in which the depth resolution is determined by the coherence length of the broadband light source. The contrast in the OCT depth scans is based on differences in backscattering properties of the tissue under study. These contrast-based reflection profiles are called Amplitude scans (A-scans). As the amplitude of the backscattered light is related to tissue-specific optical properties, tissue-distinctive A-scans are yielded. Adjacent A-scans can be merged to create two-dimensional scale cross-sectional OCT brightness scans (B-scans), (Figure 1). Based on these B-scans, OCT systems can generate three-dimensional image sets for improved spatial insight.

In general, OCT images have an axial resolution of ~10 µm. Therewith, OCT fills the gap on the resolution scale between confocal microscopy and ultrasound imaging. The amplitude of the backscattered light reduces exponentially with tissue depth. This limits the OCT imaging range to approximately 2-3 mm tissue depth.

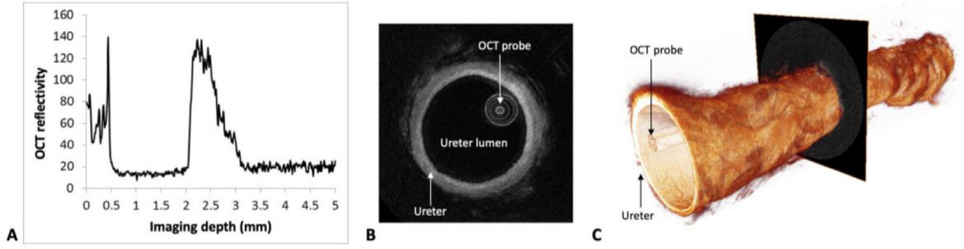

Figure 1: From A-scan to B-scan to 3D-scan; A) amplitude scan of OCT, B) brightness scan of OCT, C) three-dimensional scan of OCT.

At present, OCT has become a valuable tool for non- or minimally-invasive imaging in ophthalmology and cardiology due to its non-contact mode imaging capabilities and image resolution. OCT seems also ideal for imaging of epithelial tumours as most of them arise within the first millimetres of the superficial layers.[1] Furthermore, carcinogenesis alters the nuclear morphology, which changes the optical properties of malignant tissue. This leads to differences in OCT reflection profiles between benign and malignant tissue.[2] Hence, these OCT reflection profiles allow for qualitative and quantitative assessment for tumour diagnosis. Tissue layers can be delineated visually on OCT B-scans. Also, quantitative analyses of OCT images have been explored. Statistical, structural and spectral texture analysis of OCT images have been applied for computer-aided tumour detection.[3,4] Quantification of OCT signal depolarization may also serve for automated diagnosis.[5,6] Furthermore, the decrease in OCT signal with tissue depth, quantified by the attenuation coefficient (μ_{OCT}, mm^{-1}), has been explored for cancer diagnosis and histologic grading.[7–9]

Currently, three different types of OCT devices, interfaced to OCT systems, have been investigated for urologic applications: (1) hand-held forward-looking devices; (2) forward-looking probes of >3 Fr and (3) sideward-looking probes of <3 Fr. The hand-held device is used for extracorporeal en-face OCT imaging of epithelial tissue. Forward-looking probes are used for enface OCT imaging of tissue during endoscopic or laparoscopic procedures. Sideward-looking probes, developed for cardiovascular imaging, are compatible with flexible ureteroscopes and 18 G needles, and hence enable ureterorenoscopic or needle-based imaging of soft tissues. Characteristic for the sideward-looking probes is the automated rotary pull-back system that enables helical imaging of the peripheral surrounding during the pullback. The availability of different types of OCT devices and the variety in OCT image assessment methods facilitate possibilities to optimize the current diagnostic pathways in urologic oncology.

The aim of this comprehensive review is to provide an overview of the clinical readiness level of OCT in urologic oncology. Secondly, the diagnostic and clinical practical value of OCT for the diagnosis of malignancies in the bladder, upper urinary tract, kidneys, prostate, testes, and penis are discussed. The assessment of the clinical readiness level and the potential role of OCT is essential to guide further developments towards clinical implementation into urologic oncology.

Materials and methods

A systematic search of the literature was conducted in PubMed and Embase for each urologic cancer with key terms to identify English and German original articles on OCT in human studies until December 2017 (see Supplements). All original articles on ex- or in-vivo human studies were included. Animal studies were excluded. In case of overlapping study populations from the same group, only the most recent article was included. Reviews were excluded. Case reports were also excluded, unless the findings presented a landmark in the development of OCT.

Data extraction was performed by two independent reviewers (J.E.F., D.M.B.). In case of disagreement, consensus was reached with a third reviewer (M.B.). The results of the evidence acquisition are illustrated in Figure S1 (see Supplements).

The level of evidence (LoE) of the included articles was assessed according to the Oxford Centre for Evidence-based Medicine model.[10] Furthermore, the articles were rated according to the IDEAL recommendations to assess the clinical readiness level.[11] Table 1 illustrates the key considerations to determine the IDEAL stage as reported by Pennell et al. [11]

We did not proceed to a systematic review because of the explorative stage of OCT in urology.

Results

Bladder cancer

Bladder cancer (BC) is ranked amongst the ten most common cancers worldwide.[12] BC arises in 90% of the cases from the urothelium. Normal urothelium consists of a well-organized layer of 2-7 transitional cells with a mean layer thickness of 61 µm.[13] The urothelium is demarcated from the underlying lamina propria (LP) by the basement membrane. Underneath the LP lies the muscularis propria (MP). In a normal condition, all tissue layers of the bladder are regularly structured and well organized.[14,15] Malignant urothelium, however, has a tendency towards disorganization of the microarchitecture and the possibility of invasiveness into the underlying tissue-layers.

Histopathological assessment of BC is fundamental for diagnosis, risk-stratification, and prognosis. However, the clinical practical value and the diagnostic accuracy of transurethral biopsies or resection are limited.[16]

Especially the identification of carcinoma in situ (CIS) is challenging with white light cystoscopy.[12] Furthermore, in the absence of transurethral resection or biopsies, the diagnostic pathway for BC lacks histologic certitude for a definite diagnosis. This hampers direct outpatient treatment of low risk tumours with active surveillance or laser fulguration. Therefore, new optical imaging techniques such as OCT may lead to an improvement of the current diagnostic pathway and personalized care for BC.

Twenty relevant articles on OCT in BC were identified in the systematic search, of which two were overlapping study populations (Table 2).[1,3,22–29,5–7,17–21]

OCT imaging of human urologic tissue was performed for the first time in an ex-vivo setting in 1997.[17] Shortly thereafter, the first in-vivo OCT imaging was performed during cystoscopy with a forward-looking OCT probe. Visual delineation of tissue layers with OCT was feasible. The size of the cellular structures on OCT images corresponded with histologic morphometry of the resection specimen.[1] Further studies reaffirmed the following OCT-characteristics of bladder tissue: normal urothelium is seen as a thin horizontal layer of uniform low signal intensity. [6,18–22,26,27] The LP causes more scattering and thus appears as a brighter horizontal underlying layer with OCT. Due to the abrupt change of optical properties between the urothelium and the LP, a clear demarcation between the tissue layers can be seen in OCT images (Figure 2). Between these two layers, the basement membrane may be visualized with OCT, enhancing the demarcation between the urothelium and the LP. Interruption of this demarcation line in OCT images is characteristic for tumour invasion (Figure 2).[20,26] In OCT images of muscle-invasive BC, the layer of the LP is irregular or disrupted towards the MP.[18,19,22,26] Regardless of invasiveness, BC is usually visible as an irregular, thickened layer of heterogeneous signal intensity in OCT images. The visualization of CIS by means of OCT is difficult. In OCT images, CIS and inflammation are characterized by heterogeneous signal intensity of the unbroadened urothelium, causing a reduced contrast with the untouched LP.[19]

Several studies have described the use of OCT as adjunct to white light cystoscopy for bladder cancer diagnosis and staging.[18,19,21,22,25] In these mainly small cohorts, the sensitivity and specificity of OCT during cystoscopy for BC diagnosis ranged from 75-100% and 65-89%, respectively.[6,18,22,26,27] OCT adjunct to cystoscopy resulted in an increase in sensitivity and specificity of 19% for BC diagnosis in comparison to WL cystoscopy.[24] Schmidbauer et al. and Zagaynova et al. also illustrated an increase in the diagnostic accuracy when using OCT in conjunction with photodynamic diagnostics.[22,25]

For the diagnosis of non-muscle invasive BC, Goh et al. reported a sensitivity of 75-90% and a specificity of 89% with OCT. For the diagnosis of BC invasion, sensitivities of 100% and specificity of 77-90% were reported for OCT.[21,22]

Besides qualitative assessment, quantitative analysis of changes in OCT cross-polarization backscattering and integral depolarization factor reached a diagnostic accuracy for BC of 75%.[5,6,28] However, quantitative analysis of the μ_{OCT} was not feasible in ex-vivo for BC diagnosis and grading. The negative results were thought to be caused by the ex-vivo study design.[7]

In summary, OCT enables qualitative BC diagnosis and staging but clinical readiness level is at IDEAL stage 3 with evidence limited to level 2b. Cross-polarization OCT may enable quantitative BC diagnosis and other quantitative OCT-based analyses should be explored further. As such, OCT could facilitate real-time outpatient evaluation of suspicious bladder lesions without transurethral resection for histopathology. However, qualitative OCT-based diagnosis of CIS was not possible due to the similarity with inflammation. Future research should be stimulated to reaffirm the clinical potential and to advance quantitative algorithms for BC diagnosis. Yet, the lack of a commercially available forward-looking OCT-probe may be a threat to further development.

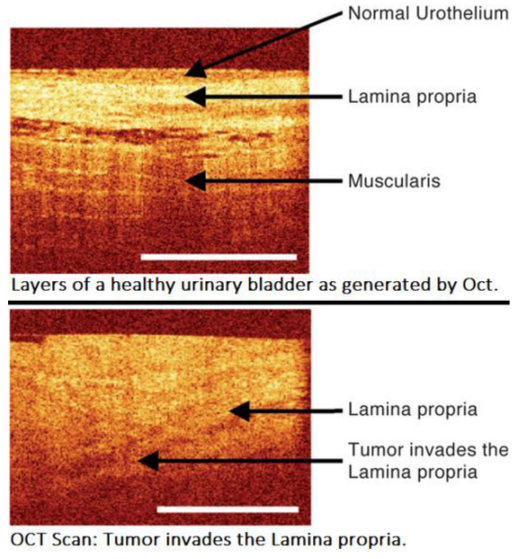

Figure 2: OCT scans of bladder cancer (published by Karl et al. in Eur. J. Med. Res. 2010).

Upper urinary tract cancer

Upper urinary tract cancers are almost exclusively upper tract urothelial carcinomas (UTUC). It is a rare disease with an incidence of about 2 cases per 100,000 people.[30] The histopathology of benign and malignant tissue in the upper urinary is highly similar to bladder urothelium. The normal intraluminal lining of the upper urinary tract consists of a few well-organized urothelial cell layers. The LP is clearly demarcated on each site by either the urothelium or the smooth muscle layer. Malignant urothelium is characterized by a tendency towards disorganization of urothelial cells and undefined tissue borders in case of invasiveness.[14]

Ureteroscopic tissue biopsies are essential for the diagnostic process. Tumour grade and stage are decisive factors in the choice between radical nephroureterectomy (RNU) or kidney sparing treatment.[30] However, ureteroscopic biopsy in the upper urinary tract is challenging due to the restricted range of motion of the instruments, the risk of complications and the restricted sampling. Together with interpretation problems due to the limited biopsy volume, fragmentation and inter-observer variability up to 20% of upper urinary tract biopsies are inconclusive. This results in limited reliability of UTUC grading and staging by ureteroscopic biopsies.[31,32]

Literature regarding the use of OCT in the upper tract is scarce. The systematic search yielded six relevant articles, of which 2 were overlapping studies.[9,17,33–35] Moreover, another two studies primarily investigated the use of OCT for renal cancer diagnostics.[34,35] An additional article that investigated OCT in normal UT and UTUC was identified by snowballing.[36] The studies that primarily investigated OCT for UTUC are presented in Table 3. Already in 1997, ex-vivo OCT imaging of the upper urinary tract was investigated.[17] Visual tissue-layer identification, as observed in the bladder, was confirmed for normal UT and invasive UTUC.[33–36]

The development the sideward-looking OCT probes for intravascular imaging has enabled the use of OCT during ureterorenoscopy.[9,36] In this way, real-time endoluminal OCT imaging enables visualization of UTUC and the layered tissue anatomy (Figure 3). Interruption and loss of anatomical layers are characteristic for invasive UTUC. Bus et al. reported a concordance of 83% for qualitative tumour staging on OCT images in comparison to the RNU histopathology.[9] In the same study, quantitative μ_{OCT} analysis lead to differentiation between low grade and high grade UTUC. A μ_{OCT} cut-off value of 2.4 mm^{-1} for the differentiation of low and high grade UTUC resulted in a sensitivity of 87% and a specificity of 90%. However, μ_{OCT} analysis was not feasible for normal urothelium and CIS. The infeasibility to measure the change of OCT signal with imaging depth might be caused by the thinness of the urothelial layer in these cases. Moreover, tumour staging was restricted to exophytic tumours of <2 mm depth due to the limited OCT imaging range.[9] Another drawback was that the sideward-looking probe requires a parallel position with the tissue of interest for OCT imaging. This may impair the visualization of the calyceal cavities with OCT. Nevertheless, this LoE 2b study at IDEAL stage 2b showed that OCT has a high diagnostic accuracy and clinical potential for perioperative UTUC grading and staging.[9] Future research should be warranted. The development of a combined sideward- and forward-looking probe may overcome current technical limitations in the application of the technique for UTUC diagnosis.

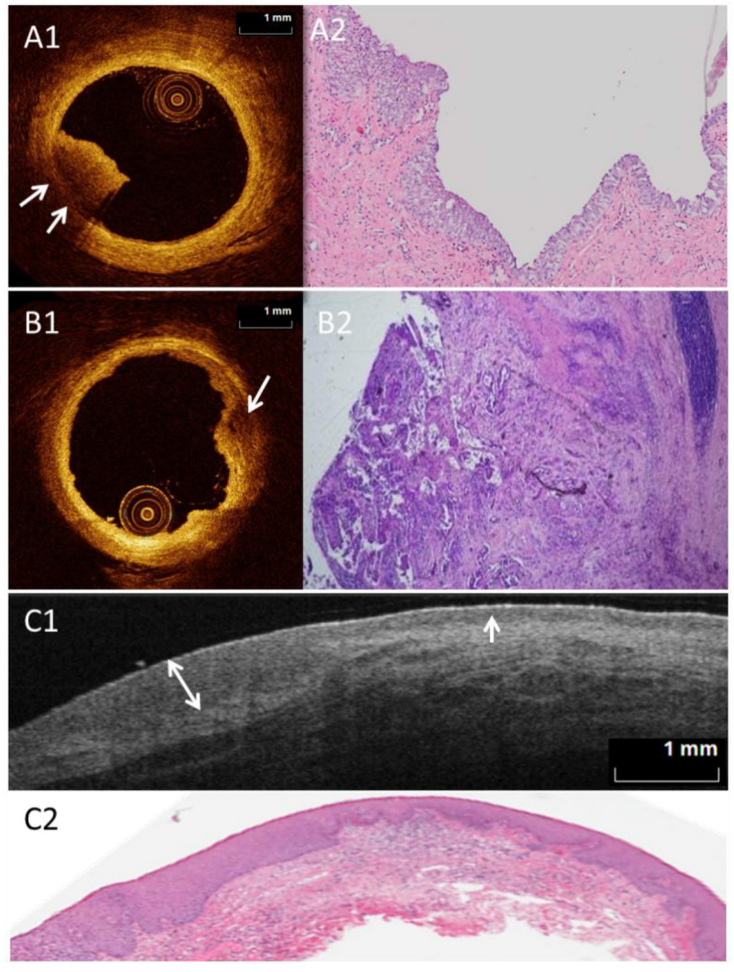

Figure 3: A1+2) Intraluminal OCT B-scan & histopathology of a stage pTa UTUC with a continuous lamina propria (arrows), B1+2) Intraluminal OCT B-scan & histopathology of a pT2 UTUC with a disrupted lamina propria & heterogeneous backscattering (arrow), C1+2) OCT B-scan & histopathology of a penile intraepithelial neoplasia with broadened epithelium (double arrow), adjacent to normal epithelium (single arrow).

Kidney cancer

Throughout the past decades we have witnessed an increase in kidney cancer, mostly at the expenses of small renal masses (SRM, < 4 cm).[37] About 90% of all kidney cancers are renal cell carcinomas (RCC), arising from the renal parenchyma. Multiple subtypes with heterogeneous clinical behaviour can be identified by histo-morphologic features, micro-anatomic origin and by molecular alterations.[38] Clear cell RCC (ccRCC) is the most common subtype accounting for 75- 80% of all RCCs. Its characteristic clear cell appearance is caused by the lipid and glycogen rich cytoplasm.[14] Papillary RCC (pRCC) with a tubulo-papillary architecture and chromophope RCC (chRCC) are found in 10% and 5% of RCC patients, respectively.[39] Other more rare RCC subtypes, invasive UTUC and benign tumours may also be the underlying pathology of renal masses.

Analysis of resected renal masses revealed 17-40% of benign pathology, mostly angiomyolipomas and oncocytomas.[40] This implies a clinically significant issue of preoperative misclassification.[40] As benign renal masses and cT1 RCC qualify for nephron-sparing treatment, preoperative renal mass biopsies (RMB) are gaining interest in the diagnostic algorithm. However, while accurate in terms of histologic reliability, RMB have a non-diagnostic rate up of to 23%, which is negatively correlated with renal mass size.[41,42] This shortcoming highlights the need for a test with a high diagnostic yield and accuracy to avoid unnecessary treatment or repeated biopsies.

Based on the systematic search, nine relevant articles were identified, of which two had overlapping populations (Table 4).[34,35,43–47] In the first ex-vivo studies, qualitative assessment of OCT images for the diagnosis of RCC was challenging, owing to the heterogeneous appearance of RCC and the lack of OCT distinctive features.[34,35,44] Qualitative differentiation was only feasible in AML, oncocytomas and invasive UTUC, where microarchitectural changes were recognizable.[34,35] AML showed characteristic features of fat, which appeared as hypodense areas (Figure 4). Oncocytomas demonstrated cystic areas and lobulated structures (Figure 4).[34,35] In another study, qualitative OCT image assessment by three independent observers yielded a range in sensitivity and specificity from 88% to 100% for the differentiation of oncocytomas from RCCs.[44]

Also, quantitative μ_{OCT} analysis has been investigated for RCC diagnosis. Studies have shown that the μ_{OCT} of RCC was significantly higher than normal renal parenchyma.[43,45,46] Buijs et al. investigated the diagnostic accuracy and yield of RMBs and concomitant in-vivo needle-based OCT in comparison to the histopathology of the resected specimen.[46] In their interim analysis cohort of 95 patients, μ_{OCT} analysis led to a diagnostic yield of 99%, compared to a

79% diagnostic yield for conventional RMBs. For differentiation between benign and malignant RMs, μ_{OCT} showed a sensitivity of 91% and a specificity of 56%. For the differentiation of RCC from oncocytoma a sensitivity of 92% and a specificity of 67% were reported.[46] Since RMB is accompanied with a high non-diagnostic rate in smaller tumours, authors suggest that OCT can potentially function as an add-on test or even as replacement test in this niche of non-diagnostic cases.

Quantitative μ_{OCT} analysis has also been evaluated for ex-vivo assessment of surgical margins during partial nephrectomies. Authors demonstrated a high accuracy for identifying positive margins (sensitivity and specificity both 100%), although the value was limited by a small sample size.[47]

As such, quantitative μ_{OCT} analysis for renal mass diagnosis has diagnostic potential. OCT adjunct to RMBs may enhance the diagnostic yield to improve the diagnostic pathway, with a possible “best benefit trade-off” in SRMs. The literature on OCT is still limited to LoE 2b at IDEAL stage 2b but respectable clinical cohort samples have been investigated.

Prostate cancer

Prostate cancer (PC) is the most common cancer in men.[48] In 95% of the cases, PC is an adenocarcinoma. Other types of PC are small cell carcinomas, urothelial carcinomas, squamous or basal cell carcinomas.[14] In the normal prostate gland non-glandular and glandular tissues are interbred. Normal glandular tissue consists of organized stroma with smooth muscle fibers and well-organized glandular acini and ducts. Normal acini and ducts share a well-established microarchitecture of secretory, basal and neuroendocrine cells. Adenocarcinomas originate in 80% of the cases from acini or ducts.[14] Malignancy is characterized by a tendency towards disorganization, loss of architecture and lack of basal cells. The Gleason-score and the corresponding grade groups are used to evaluate the grade of malignancy based on the architectural and morphologic alterations.[49] Prostate biopsies are the reference standard for PC diagnosis. However, the accuracy of tumour localization and demarcation of the current diagnostic approach is limited due to the multifocal nature of PC. Especially in the scope of focal therapies, exact identification of prostate cancer foci is required, driving the interest in new imaging modalities.

Six relevant articles on OCT in PC were identified with the systematic search, of which three use overlapping populations (Table 5).[8,50–52] The first OCT on human prostates was performed in an ex-vivo study in 2000.[50] OCT allowed demarcation of the prostatic microarchitecture.[50,52] Visualization of

irregular gland contours, the infiltration of benign glands or the visualization of cribriform patterns were characteristic for PC on OCT images, demonstrating a sensitivity and specificity of 63% and 74%, respectively.[47] However, micro-focal PC may be missed with OCT, although the clinical implication of these foci is unknown.[52]

In 2016, the feasibility of quantitative μ_{OCT}-based PC diagnosis was investigated in prostatectomy specimen with the sideward-looking OCT probe in a needle-based fashion.[8] In this study, a customized tool for histopathology slicing was used for exact co-localization of the histopathology with OCT images (Figure 4). Despite the limited sample size, the μ_{OCT} in PC was significantly higher than in benign prostate tissue.[8]

OCT was also investigated for the assessment of surgical margins after robotic prostatectomy in 100 patients.[51] In this study, OCT-based predictions for the assessment of surgical margins were compared to the histopathology of the radical prostatectomy, yielding a sensitivity of 70% and a specificity of 84%.[51] OCT characteristics for positive margins were seen as heterogeneous columns of low signal intensity extending from the subepithelium to the surface of the serosa.

On balance, OCT has reached LoE 2b at an IDEAL stage 2b in ex-vivo studies, where visual assessment of OCT images and quantitative μ_{OCT} analysis allow for PC identification.12 Yet. translation to in-vivo studies for PC diagnosis is lacking, possibly because the additional value of OCT for PC diagnosis may be marginal when considering the improvements of radiology guided biopsies. A possible role for OCT in PC might be intraoperative assessment of surgical margins during radical surgery.

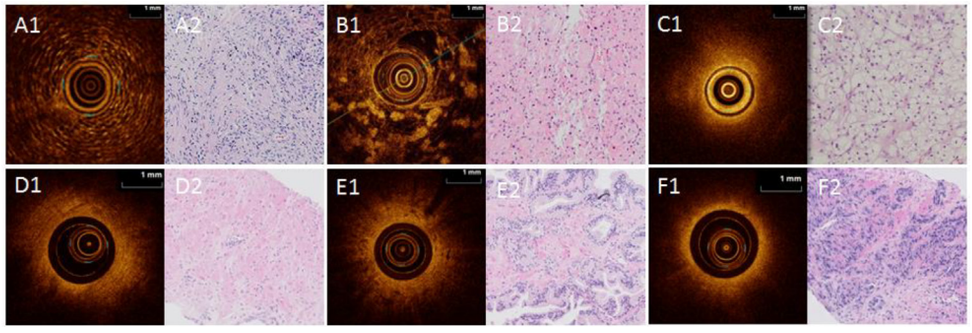

Figure 4: A1+2) Needle-based OCT B-scan & histopathology of renal angiomyolipoma, B1+2) Needle-based OCT B-scan & histopathology of renal oncocytoma, C1+2) Needle-based OCT B-scan & histopathology of clear cell RCC, D1+2) Needle-based OCT B-scan & histopathology of prostate stroma, E1+2) Needle-based OCT B-scan & histopathology of benign glandular prostate, F1+2) Needle-based OCT B-scan & histopathology of prostate cancer Gleason 4+4.

Testicular cancer

Testicular cancer is the most prevalent solid cancer in young men. In 95% of the cases, testicular cancers are germ cell tumours, mostly seminomas and non-seminomas.[14] Ultrasound of the scrotum is the index test for initial diagnosis. In case of suspicious tumours, inguinal orchiectomy is diagnostic and therapeutic. Testicular biopsies are not considered in the presence of a normal contralateral case due to the risk of tumour seeding. According to our systematic search, OCT has not been investigated in testicular cancer. A hypothetical use of OCT in testicular tumours is the perioperative assessment of resection margins in partial orchiectomy.

Penile cancer

Penile cancer (PeC) is a rare disease in the Western World. It usually arises from malignancy of the epithelium of the glans or the prepuce. PeC is almost exclusively squamous cell carcinomas (SCC), although it may exhibit different morphologies. PeC presents as superficial horizontal growth, superficial exophytic growth or as invasive growth into deeper tissue layers.[14] SCC may be preceded by penile intraepithelial neoplasia. This pre-malignancy is characterized by a disorganized basal layer of the epithelium.[14] Punch biopsies are the reference standard for diagnosis.

Only two relevant articles were identified from the systematic search. A case report described the first use of OCT in premalignant penile lesions. The continuous dermo-epidermal junction zone of the superficial premalignant lesion was visualized and the local chemotherapy response was monitored.[53] A prospective cohort study with LoE 2b at IDEAL stage 2a evaluated the use of OCT for the diagnosis of suspicious penile lesions (Table 6). OCT images of suspicious penile lesions were compared to histopathology of punch biopsies in 18 patients. Besides qualitative assessment of the integrity of tissue layers, quantification of the epidermal thickness and μ_{OCT} analysis enabled PeC diagnosis (Figure 3).[54] Despite the limited evidence of this single study of 2b LoE, OCT seems to have a promising diagnostic potential with a high clinical practical value for PeC and penile intraepithelial neoplasia.

Discussion

With regard to the diagnostic potential of OCT in urologic oncology, the present appraisal on the clinical readiness level of OCT shows an overall LoE 2b and up to 2b-3 IDEAL stages for all major urologic cancers with the exception of testis cancer. Overall, the current evidence indicates that OCT holds the potential as a replacement or add-on test for the diagnostic pathways in BC, UTUC, RCC and PeC. However, the setting is still exploratory as studies are predominantly

single centre studies with restricted and heterogeneous samples, OCT methodology and technologic application. In some uro-oncologic diseases, the technical level has not yet met all clinical requirements, and optimized OCT systems for urologic applications are still lacking. Nevertheless, in the light of some promising results and potential clinical practicality, further research should be fuelled on OCT in most urologic oncology conditions. Future research and clinical implementation of OCT will eventually depend on thorough appraisal of the potential diagnostic gains, clinical availability and costs in comparison with the index tests. Notably, we should catch the wave to combine different optical imaging techniques for a multimodal optical biopsy approach. Multimodal optical assessment, especially in combination with computer-aided diagnosis, could boost the additional diagnostic value of the new techniques with regard to the conventional diagnostic paradigms.

In BC, transurethral resection is concomitantly of diagnostic and therapeutic use. As such, OCT may only be of an additional value during cystoscopy to provide histologic certitude for discharge, continuing routine follow-up, expectative policy, or outpatient laser vaporization of low risk BC. However, the clinical readiness level of OCT is hampered by the lack of a commercially available forward-looking probe.

In UTUC, the potential of OCT as an add-on or even replacement test for ureteroscopic biopsies seems alluring. OCT holds a high clinical and practical value for UTUC diagnosis because of its real-time character and the limited diagnostic yield and clinical burden of ureteroscopic biopsies. Especially, quantitative tumour grade differentiation could provide practical and objective intraoperative risk-stratification for adequate selection and immediate ureteroscopic treatment. Therefore, validation studies and phase III trials as well as technical development of a combined sideward- and forward-looking probe should be encouraged. Yet, the cost-effectiveness may be at risk as UTUC is a rare disease.

Within the scope of the ongoing discussion on the role of RMB, especially in SRMs, OCT could be applied as an add-on test to improve the diagnostic pathway. Further research to validate quantitative outcomes for μ_{OCT}-based diagnosis should be undertaken. Again, one should carefully balance the value of this add-on benefit versus the additional costs. Moreover, OCT could be used for intra-operative assessment of surgical margins in partial nephrectomies.

In PeC, OCT could serve as a non-invasive replacement test for punch biopsies of suspicious penile lesions. Furthermore, OCT might even enable monitoring of the topical treatment response in penile intraepithelial neoplasia. Despite the low incidence of PeC, further research should be stimulated to evaluate its diagnostic value. In view of the frequent centralized care of PeC and the use of re-usable handheld OCT devices, the cost-effectiveness of OCT for PeC could be reasonable.

The role of OCT for PC diagnosis or image guidance adjunct to prostate biopsies seems limited in additional diagnostic value. The combination of radiologic imaging with systematic and targeted biopsies represent a fierce competitor for optical imaging in PC diagnosis.[55] Yet, OCT could provide real-time histologic information. However, translation to in-vivo diagnostic studies is lacking. Only one in-vivo study for OCT-based assessment of resection margins in prostatectomies has been performed. Future research should define the role of OCT in PC.

At the present, OCT information for the diagnosis of testicular cancer is lacking. Due to the fact that diagnostic biopsies are not indicated in testicular cancer, the role of OCT will be limited and at the most may be useful for perioperative assessment of the surgical margins during partial orchiectomy.

Overall, the strength of this comprehensive review is the systematic elaboration of the current clinical readiness level of OCT in all major uro-oncologic diseases. However, the lack of high-level evidence, as well as the heterogeneity of study populations and technical data limit the feasibility of a sound systematic review. Hence, conclusions are based on the first stages of evaluation of a new technology. However, thorough evaluation is mandatory before clinical implementation. Moreover, early identification of conditions in which OCT may be most beneficial may stimulate and catalyse future developments. As such, this review will guide further translational research towards the implementation of OCT into uro-oncologic practice.

Table 1: Overview of key considerations used to determine the “Idea, Development, Exploration, Assessment & Long-term follow-up (IDEAL) stages as reported by Punnell et al. in the British Journal of Surgery in 2016.

IDEAL stage	Characteristics of reports	Key issues addressed and content items	Key milestones for stage completion
Idea (1)	One or very few reports. Only case reports or very small case series.	States that this is first in humans. Detailed technical description.	Reports an intervention not previously used in humans.
Development (2a)	Small number of reports. Reports from one or a few centres. All reports have small number of patients	Safety of procedure. Short-term outcomes. Discusses indications, technical detail and may describe modifications.	Content and nature of reports suggest intervention technique has reached stability.
Exploration (2b)	Increasing number of reports, patients per report and centres involved. Some prospective collaborative studies (registries, audits, databases).	Discusses procedural quality and learning curves. Comparison of outcomes with standard treatment. Calls for an RCT to be done.	Reports suggest that consensus has been reached on optimal technique, indications and outcome measures.
Assessment (3)	Reports of multicentre RCTs. Quasi-experimental designs. Stepped-wedge designs. Case-matching studies. Analysis of large data sets with risk adjustment.	Compares procedure with standard treatment.	Reports document a high-quality RCT or other valid experimental comparison of the intervention compared with the current standard of care.
Long-term (4)	Long-term cohort studies. Retrospective case series. Registries and databases.	Reports long-term outcomes. Identifies rare outcomes. May analyse risk or prognostic factors. May report on changing indications.	Ongoing reports of Late or rare outcomes Which patients benefit most Whether indications are changing Variation in performance

Table 2: Overview of studies on OCT for human bladder cancer.

Author, *year*	Study type	Study aim	Sample size (ROI)	OCT system	Reference standard	Findings of qualitative OCT assessment	Findings of quantitative OCT analysis	LoE	IDEAL stage
Kiseleva et al, 2017	Prospective cohort, ex-vivo	Assessing CP OCT for BC	18 (60)	CP OCT, AR na, 1310nm λ	Cystectomy specimen	In mild inflammation the urothelium is visualized as a continuous layer of high CP OCT signal intensity, while in severe inflammation, the OCT signal intensity is lower. In poorly differentiated invasive BC, the urothelium is structureless with low OCT signal intensity.	Depolarization factor maps and integral depolarization factor analysis enabled differentiation of BC from inflammation.	3b	2a
Kiseleva et al, *2015*	Prospective cohort, in-vivo	Assessing computer-aided CP OCT analysis for BC	73 (96)	CP OCT 1300U, 8Fr FL probe, 20µm AR, 1315nm λ	Biopsy	NP	Computer aided integral depolarization factor analysis enabled differentiation of BC from inflammation with a diagnostic accuracy was 75%. Recurrence in the post-operative scar could be identified with this technique in 97% of the cases.	2b	2b
Gladkova et al, *2013*	Prospective cohort, in-vivo	Assessing CP OCT in comparison to OCT and fluorescence cystoscopy	26 (92)	CP OCT 1300-U, 8Fr FL probe, 15µm AR, 1315nm λ	Biopsy	Visual assessment of traditional and CP OCT images as an add-on to fluorescence cystoscopy improved the diagnostic accuracy for CIS diagnosis. The best results were reached with CP OCT as add-on, resulting in a sensitivity of 90% and specificity of 92% for CIS.	The quantitative estimation of the CP OCT signal intensity, expressed as standard deviations in dB, differed significantly in benign states from CIS and BC. 4.32 dB was taken to be the threshold for diagnosing CIS. This yielded a sensitivity of 96% with a specificity of 92% for CIS detection in combination with fluorescence cystoscopy.	2b	2a
Gladkova et al, *2011*	Prospective cohort, in-vivo	Comparing traditional OCT with CP OCT for BC diagnosis	116 (360)	CP OCT, 8Fr FL probe, 15µm AR, 1300nm λ	Biopsy	CP OCT image assessment lead to a sensitivity of 94% and specificity of 84% in detecting CIS. Visual interpretation of traditional OCT images resulted in a significantly lower sensitivity (81%) and specificity (70%).	NP	2b	2b
Cauberg et al, *2010*	Prospective cohort, ex-vivo	Assessing μ_{OCT} analysis for BC grading	18 (54)	Time domain OCT, 14µm AR, 850nm λ	Resection chips	NP	μ_{OCT} analysis did not predict BC tumour grade. The median μ_{OCT} of benign tissue was 5.75 mm^{-1} vs 5.52mm^{-1}, 4.85 mm^{-1}, and 5.62 mm^{-1} for grade 1, 2, and 3 BC, respectively.	2b	2a
Karl et al, *2010*	Prospective cohort, in-vivo	Assessment of OCT for BC diagnosis	52 (102)	Niris OCT, 8Fr FL probe, 20µm AR, 1310nm λ	Biopsy or resection chips	Sensitivity and specificity for visual BC diagnosis with OCT was 100% and 65% respectively. The sensitivity for detection of tumour growth beyond the lamina propria was 100%.	NP	2b	3
Schmidbauer et al, *2009*	Prospective cohort, in-vivo	Assessment of OCT adjunct to WL or fluorescence cystoscopy	66 (232)	Niris OCT, 8Fr FL probe, 15µm AR, 1310nm λ	Biopsy	Combining fluorescence cystoscopy with targeted OCT increased the specificity significantly. Sensitivity and specificity for BC detection with WL cystoscopy were 69.3% and 83.7%, with fluorescence cystoscopy 97.5% and 78.6% and with fluorescence cystoscopy adjunct to targeted OCT 97.5% and 97.9%.	NP	2b	3
Ren et al, *2009*	Prospective cohort, in-vivo	Assessment of OCT for BC diagnosis	56 (110)	Spectral-domain OCT, 8Fr FL probe, 10µm AR, 1320nm λ	Biopsy or resection chips	Sensitivity of OCT for BC detection was significantly higher (94%) than cystoscopy alone (75%) and voided cytology (59%). Specificity of OCT (81%) was comparable to voided cytology (88.9%), but significantly higher than for cystoscopy (62.5%).	NP	2b	3
Goh et al, *2008*	Prospective cohort, in-vivo	Assessment of OCT for intra-operative BC staging	32 (38)	Niris OCT, 8Fr FL probe, 15µm AR, 1310nm λ	Biopsy or resection chips	OCT enabled correct staging in 18/20 Ta BC. OCT enabled correct identification of invasion into the lamina propria in 11/11 ≥T1 BC. OCT enabled correct staging in 7/7 MIBC.	NP	2b	3
Lingley-Papadopoulos et al, *2009*	Post-hoc analysis of two prospective cohorts, in-vivo	Evaluation of two texture analysis algorithms for OCT based cancer detection	Training set: 22 (196) Test set: 34 (96)	Imalux Corporation OCT, 8Fr FL probe, AR na, test set 1310nm λ, training set 980nm λ	Biopsy	NP	With images from the same OCT system, the OCT system dependent algorithm for texture analysis yielded a sensitivity of 73% and specificity of 69% for BC, but the algorithm failed with images from a different OCT system. Regardless of the OCT system, the OCT system independent algorithm for texture analysis demonstrated a sensitivity of 87% and specificity of 58% for BC diagnosis.	2b	2b
Hermes et al, *2008*	Prospective cohort, ex-vivo	Defining OCT parameters for BC diagnosis	na (142)	Sirius 713 OCT, FL probe, 3µm AR, 800nm λ	Resection chips, cystectomy	The basement membrane was identified as a continuous layer of minimum signal intensity between the urothelium and lamina propria in normal urothelium and non-invasive BC. This membrane allows for reliable exclusion of tumour invasion. Visual assessment of OCT yielded a sensitivity of 83% and specificity of 78% for BC diagnosis.	NP	2b	2a
Sengottayan et al, 2008	Prospective cohort, in-vivo	Evaluation of intraoperative BC staging with OCT	32 (38)	Niris OCT, 8Fr FL probe, 15µm AR, 1310nm λ	Biopsy or resection chips	OCT-based staging of Ta BC yielded a sensitivity of 90% and a specificity of 89%. In T1 BC, OCT-based staging yielded a sensitivity of 75% and a specificity of 97%. In MIBC, OCT-based staging resulted in a sensitivity of 100% and a specificity of 90%.	NP	2b	2b
Daniltchenko et al, *2006*	Prospective cohort, in-vivo	Defining OCT parameters for BC diagnosis	50 (326)	Sirius 713 OCT, FL probe, AR na, 1300nm λ	Biopsy or resection chips	Inflammatory urothelium is depicted by a continuous, thickened layer of reduced signal intensity with blurring of stratification. Ta BC is characterized by a thickened, irregular but continuous layer of increased signal intensity. MIBC is characterized by loss of stratification and heterogeneous OCT signal.	NP	2b	2a
Manyak et al, *2005*	Prospective cohort, in-vivo	Assessment of OCT for visualization of epithelial and subepithelial anatomic structures	24 (87)	Imalux Corporation OCT, 8Fr FL probe, 10-20µm AR, 980nm λ	Biopsy	Benign bladder tissue is characterized by three stratified layers: U of uniform thickness; LP as a bright, thin continuous layer and MP as darker layer. NMIBC is visualized as stratified layers but with low contrast between the U and LP, where the continuous LP is poorly defined or with focal disorganization. MIBC appeared as disorganized U with disrupted LP and poor differentiation between layers. Sensitivity and specificity for BC diagnosis were 100% and 89%.	NP	2b	2a
Zagaynova et al, *2008*	Prospective cohort, in-vivo	Assessing flat lesions with OCT and OCT-guided TURB	80 (114)	OCT, 8Fr FL probe, AR na, 1300nm λ	Biopsy or resection chips	OCT yielded a sensitivity of 85% and specificity of 68% for the diagnosis of CIS. 79% of false positive fluorescent lesions showed typical benign OCT images. OCT-based assessment of the resection line yielded a sensitivity of 93% and specificity of 74% for the identification of irradical resection margins.	NP	2b	2a
Jesser et al, 1999	Case series, ex-vivo	Feasibility study of OCT for BC	8 (na)	AFC OCT, 18µm AR, 1310nm λ	Autopsy or resection chips	OCT of normal bladder tissue enabled delineation of tissue layers: mucosa, submucosa and muscle layers. In MIBC, the delineation of tissue layers was not possible.	NP	4	1
Sergeev et al, 1997	Case series, in-vivo	Feasibility study of endoscopic OCT	3 (na)	OCT, 8Fr FL probe, AR na, 830nm λ	Resection	Visual tissue layer identification of the urothelium, LP and muscle was feasible. The sizes of cellular structures in OCT images were in concordance with sizes in resection specimen.	NP	4	1
Tearney et al, *1997*	Case series, ex-vivo	Feasibility study of endoscopic OCT	5 (na)	OCT, 16µm AR, 1300nm λ	Autopsy	Visual identification of the mucosa, submucosa and the muscularis propria of the bladder. Muscular layers had a higher OCT signal intensity than mucosal and adventitial layers.	NP	5	1

Table legends: λ = wavelength, μ_{OCT}= attenuation coefficient, AR = axial resolution, BC = bladder cancer, CIS = carcinoma in situ, CP = cross-polarization, FL = forward-looking, IDF = integral depolarization factor, LoE = level of evidence, LP = lamina propria, MIBC = muscle invasive bladder cancer, na = not available, NMIBC = non-muscle invasive bladder cancer, NP = not performed, ROI = region of interest, U = urothelium, WL = white light.

Table 3: Overview of studies on OCT for human upper tract urothelial carcinoma.

Author, *year*	Study type	Study aim	Sample size (ROI)	OCT system	Reference standard	Findings of qualitative OCT assessment	Findings of quantitative OCT analysis	LoE	IDEAL stage
Bus et al, *2016*	Prospective cohort, in-vivo	Assessment of ureteroscopic OCT for UTUC diagnosis	26 (26)	Ilumien OCT, 2.7Fr Dragonfly sideward-looking probe, 15µm AR, 1300nm λ	RNU	OCT enabled visual staging of UTUC with 83% concordance with the final histopathology. Sensitivity and specificity for tumour invasion was 100% and 92%. Exophytic tumour growth greater than 2 mm and inflammation caused false-positives.	A μ_{OCT} cut-off of 2.4 mm^{-1} yielded a sensitivity of 87% and specificity of 90% for differentiation of low and high grade UTUC. μ_{OCT} analysis was not feasible for normal urothelium and CIS.	2b	2b
Mueller-Lisse et al, 2016	Prospective cohort, in-vivo	Delineation of the upper urinary tract wall layers	12 normal UT, 3 UTUC (na)	M2 OCT-system, 400µm sideward-looking probe, 15µm AR, 1300nm λ	Ureteroscopic biopsies	In-vivo OCT is feasible and enables wall delineation of normal and malignant upper urinary tracts.	NP	2b	2a
Ikeda et al, *2013*	Case report, ex-vivo	Feasibility study for UTUC diagnosis	1 (1)	3D UHS forward-looking OCT, 23µm AR, λ na	RNU	Visual differentiation of the tissue layers in the normal ureter. Lack of structural layers in muscle-invasive UTUC.	NP	5	1
Tearney et al, *1997*	Case series, ex-vivo	Feasibility study of OCT in normal post-mortem ureters	5 (na)	OCT, 16µm AR, 1300nm λ	Post-mortem resection	Visual differentiation of the mucosa, muscular layers and adventitia of the normal ureter. The muscular layers had a higher and more regular backscattering intensity than mucosal and adventitial layers.	NP	5	1

Table legends: μ_{OCT} = attenuation coefficient, λ = wavelength, AR = axial resolution, LoE = level of evidence, na = not available, RNU = radical nephroureterectomy, ROI = region of interest, UTUC = upper tract urothelial carcinoma, UT = upper tract.

Table 4: Overview of studies on OCT for human kidney cancer.

Author, *year*	Study type	Study aim	Sample size (ROI)	OCT system	Reference standard	Findings of qualitative OCT assessment	Findings of quantitative OCT analysis	LoE	IDEAL stage
Ludwig et al, *2017*	Prospective cohort, ex-vivo	Evaluation of OCT for PN margin assessment during	15 (na)	Diagnostic Photonics OCT, handheld probe, <20µm AR, λ na	PN	Measuring the margin widths with OCT were in concordance with the histopathological margin width assessment	The median μ_{OCT} of tumour was significantly higher than adjacent normal renal parenchyma (5.43 vs 3.78 mm^{-1}). All 15 PN cases had negative margins on both OCT analysis and final pathology	2b	2a
Buijs et al, *2017*	Prospective cohort, in-vivo	Assessment of μ_{OCT} of needle-based OCT for diagnosis of RMs	95 (95)	Ilumien OCT, 2.7Fr sideward-looking probe, 15µm AR, 1300nm λ	RN, PN or second biopsy	NP	The median μ_{OCT} of oncocytoma was 3.38 mm^{-1}. The median μ_{OCT} of RCC was significantly higher (4.37 mm^{-1}). Sensitivity and specificity for μ_{OCT} differentiation between benign and malignant RMs were 92% and 67%. The diagnostic yield of needle-based OCT was 99%.	2b	2b
Jain et al, *2015*	Prospective cohort, ex-vivo	Assessment of visual analysis for diagnosis of RMs	20 (20)	FF OCT light-CT scanner, 0.8µm AR, λ na	RN	RCC showed heterogeneous appearances on OCT images but reliable qualitative differentiation was challenging. Invasive UTUC and AML were distinguishable on OCT images.	NP	2b	2a
Barwari et al, *2012*	Prospective cohort, in- & ex-vivo	Assessment of μ_{OCT} for diagnosis of RMs	16 (16)	Santec Innervision 2000; 7Fr sideward-looking probe, 9µm AR, λ na	RN	NP	The median μ_{OCT} of normal renal parenchyma was significantly lower than of malignant tumours (5.0 vs 8.2 mm^{-1}).	2b	2a
Lee et al, *2012*	Prospective cohort, ex-vivo	Feasibility study of OCT for the assessment of kidney morphology	19 (35)	Prototype integrated OCT; 4µm AR, λ na	RN	By visual assessment, three pathologists achieved a sensitivity and specificity from 88% to 100 for differentiation of benign and malignant RMs with substantial interobserver agreement.	NP	2b	2a
Linehan et al, *2011*	Prospective cohort, ex-vivo	Feasibility study of OCT to characterize RMs	20 (38)	Laboratory OCT system; 4µm AR, 890nm λ	RN	RCC and subtypes of RCC lacked anatomical elements in comparison to normal parenchyma. Invasive UTUC, oncocytoma and AML show distinctive appearances on OCT images.	NP	2b	2a
Barwari et al, *2011*	Prospective cohort, ex-vivo	Feasibility of μ_{OCT} for diagnosis of RMs	18 (26)	Santec Innervision 2000 OCT; 7Fr sideward-looking probe, 9µm AR, λ na	RN	NP	Mean μ_{OCT} of normal renal tissue (4.95 mm^{-1}) significantly lower than μ_{OCT} of RCC (4.95 vs 8.86 mm^{-1}).	2b	2a

Table legends: μ_{OCT} = attenuation coefficient, λ = wavelength, AR = axial resolution, LoE = level of evidence, na = not available, PN = partial nephrectomy, RCC = renal cell carcinoma, RM = renal mass, RN = radical nephrectomy, ROI = region of interest, UTUC = upper tract urothelial carcinoma.

Table 5: Overview of studies on OCT for human prostate cancer.

Author, *year*	Study type	Study aim	Sample size (ROI)	OCT system	Reference standard	Findings of qualitative OCT assessment	Findings of quantitative OCT analysis	LoE	IDEAL stage
Muller et al, *2016*	Prospective cohort, ex-vivo	Feasibility study of needle-based OCT for prostate cancer	6 (na)	Ilumien OCT, 2.7Fr sideward-looking probe, 10µm AR, 1300nm λ	RP with slicing tool for one-to-one matching with OCT images	NP	Prostate cancer detection based on the µoct was feasible. The μ_{OCT} for benign prostate tissue was significantly lower than in malignant prostate tissue (3.56 vs 3.85 mm^{-1}).	2b	2a
Lopater et al, *2015*	Prospective cohort, ex-vivo	Assessment of OCT-based cancer detection in prostate biopsies	38 (119)	Full Field OCT system, forward-looking probe, AR na, λ na	Systematic core biopsy	Sensitivity of 63% and specificity of 74% for visual cancer detection in core biopsies with OCT. Micro-focal prostate cancer may be missed with OCT.	NP	2b	2b
Dangle et al, *2009*	Prospective cohort, ex-vivo	Feasibility study to assess surgical margins & extra capsular extension in RP	100 (na)	Niris OCT, 8 Fr forward-looking probe, 10-20µm AR, 1310nm λ	RP	Identifying positive margins after radical prostatectomy with OCT yielded a sensitivity of 70% and specificity of 84%. Heterogeneous, low scattering columns on OCT images characterized areas of tumour invasion.	NP	2b	2a
D'Amico et al, *2000*	Prospective cohort, ex-vivo	Characterizing prostate carcinoma in OCT images	7 (na)	Forward looking OCT, 1 µm AR, 800nm λ	RP	OCT can visualize the microarchitecture in benign and malignant prostate tissue up to an imaging depth of 0.5 mm. Prostate cancer was characterized by smaller glandular lumens than in benign tissue.	NP	3b	1

Table legends: μ_{OCT} = attenuation coefficient, λ = wavelength, AR = axial resolution, LoE = level of evidence, NP = not performed, ROI = region of interest, RP = radical prostatectomy.

Table 6: Studies on OCT for human penile carcinoma.

Author, *year*	Study type	Study aim	Sample size (ROI)	OCT system	Reference standard	Findings of qualitative OCT assessment	Findings of quantitative OCT analysis	LoE	IDEAL stage
Wessels et al, *2015*	Prospective cohort, in-vivo	Feasibility study for diagnosis of (pre)malignant penile lesions	18 (18)	Santec Inner Vision 2000 forward-looking OCT, 10µm AR, 1300nm λ	Punch biopsy	Visual assessment of tissue layers, served to discriminate between benign and malignant lesions. Mean epidermal thickness measured on OCT images of benign lesions was 0.18 mm versus 0.53 mm in (pre)malignant lesions (p = 0.001).	Mean μ_{OCT} of benign lesions was significantly lower than in (pre)malignant lesions (2.5 vs 5.2 mm^{-1}).	2b	2a
Schmitz et al, *2014*	Case report, in-vivo	Feasibility study of OCT in premalignant penile lesions	1	Vivosight multibeam forward-looking OCT, 10µm AR, λ na	Punch biopsy	The dermo-epidermal junction zone can be visualized to determine invasiveness. Local treatment response can be evaluated visually with OCT.	NP	5	1

Table legends: μ_{OCT} = attenuation coefficient, λ = wavelength, AR = axial resolution, LoE = level of evidence, NP = not performed, ROI = region of interest.

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Supplements

An individual search was performed for each organ in PubMed and Embase for English and German articles up to December 2017. For each search, the key terms for OCT {OR} were combined {AND} with organ specific key terms {OR}:

- Optical coherence tomography (Mesh), Optical coherence tomography, OCT
- Urinary Bladder Neoplasms (Mesh), Bladder neoplasm, Bladder tumor, Bladder cancer, Bladder carcinoma, Bladder malignancy
- Urothelial carcinoma AND {Ureter OR Renal pelvis}, Transitional cell carcinoma AND {Ureter OR Renal pelvis}, Upper urinary tract tumor, UTUC, Urinary tract tumor, Urinary tract cancer, Pelvis tumor, Ureter tumor
- Renal Cell Carcinoma (Mesh), Renal neoplasm, Renal tumor, Renal mass, Renal cancer, Renal carcinoma, Renal malignancy, Kidney neoplasm, Kidney tumor, Kidney mass, Kidney cancer, Kidney carcinoma, Kidney malignancy
- Prostatic Neoplasms (Mesh), Prostate tumor, Prostate cancer, Prostate malignancy
- Testicular Neoplasms (Mesh), Testicular tumor, Testicular cancer, Testicular carcinoma, Testicular malignancy, Testis neoplasm, Testis tumor, Testis cancer, Testis carcinoma, Testis malignancy
- Penile Neoplasms (Mesh), Penile tumor, Penile cancer, Penile carcinoma, Penile malignancy, Penis neoplasm, Penis tumor, Penis cancer, Penis carcinoma, Penis malignancy

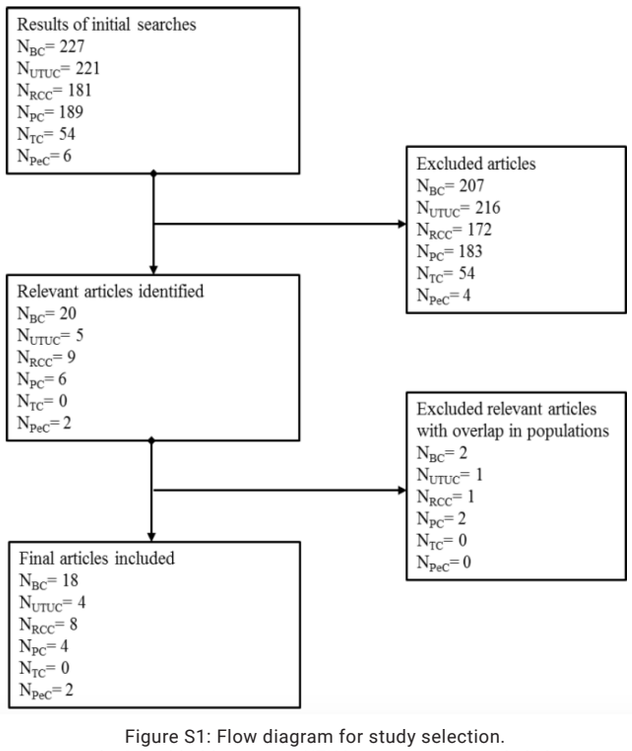

Figure S1: Flow diagram for study selection.
Figure Legends: BC = bladder cancer, UTUC = upper tract urothelial carcinoma, RCC = renal cell carcinoma, PC = prostate cancer, TC = testicular cancer, PeC = penile cancer.

Chapter 5

Grading Upper Tract Urothelial Carcinoma with the Attenuation Coefficient of in-vivo Optical Coherence Tomography

J.E. Freund, D.J. Faber, M.T. Bus, T. G. van Leeuwen, D.M. de Bruin
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List of abbreviations

A-scan – amplitude scan
AUC – area under the curve
IQR – interquartile range
μ_{OCT} – optical coherence tomography attenuation coefficient
OCT – optical coherence tomography
ROC – receiver operating characteristic
ROI – region of interest
UTUC – upper tract urothelial carcinoma
WHO – World Health Organization

Abstract

Introduction: With catheter based optical coherence tomography (OCT), high resolution images of the upper urinary tract can be obtained, thereby facilitating the detection of upper tract urothelial carcinomas (UTUC). We hypothesized that the attenuation coefficient of the OCT signal (μ_{OCT}) will be related to the histopathologic grade of the tumour.

Objectives: In this study, we aimed to define the μ_{OCT} cut-off for discriminating high grade and low grade papillary UTUC.

Methods: For this post-hoc analysis, data from OCT imaging of papillary UTUC was obtained from patients during ureterorenoscopy. OCT images and raw data were simultaneously analysed with in-house developed software. The μ_{OCT} determined in papillary UTUCs and corresponding histopathologic grading from either biopsies or radical resection specimens were compared.

Results: Thirty-five papillary UTUC from 35 patients were included. μ_{OCT} analysis was feasible in all cases. The median μ_{OCT} was 3.3 mm^{-1} (IQR 2.7 – 3.7 mm^{-1}) for low-grade UTUC and 4.9 mm^{-1} (IQR 4.3 – 6.1 mm^{-1}) for high-grade UTUC ($p=0.004$). ROC analysis yielded a μ_{OCT} cut-off value of >4.0 mm^{-1} (AUC=0.85, $p<0.001$) with a sensitivity of 83% and a specificity of 94% for high-grade papillary UTUC.

Conclusions: This study proposes a μ_{OCT} cut-off of 4.0 mm^{-1} for quantitative grading of UTUC with ureterorenoscopic OCT images. The promising diagnostic accuracy calculations justify further studies to validate the proposed cut-off value. Implementation of the software for the μ_{OCT} analysis in OCT systems may allow for μ_{OCT}assessment at real time during ureterorenoscopy.

Introduction

Upper urinary tract urothelial carcinoma (UTUC) is the malignancy of the urothelial lining in the ureter and pyelocaliceal system. In Western countries, the annual incidence of UTUC is estimated at two cases per 100,000 inhabitants with a peak incidence in the elderly populations (>70 years).[1] Besides multiple etiological factors, such as smoking, UTUC is linked to hereditary nonpolyposis colorectal cancer (Lynch syndrome) in some patients.

Macroscopically, papillary UTUCs are exophytic papillary tumours (video 1, see: https://onlinelibrary.wiley.com/page/journal/10969101/homepage/lsm-23079-video001.htm).

Microscopically, these tumours are characterized by their papillary configuration of urothelial cells, as illustrated in Figure 1. The aggressiveness of UTUC is categorized as low-grade or high-grade according to the WHO classification.[2,3] High-grade is characterized by major atypia of urothelial cells and major architectural disorganization, reflecting higher biological potential.[3] Moreover, high-grade UTUC are more frequently invasive into the underlying muscle.[4]

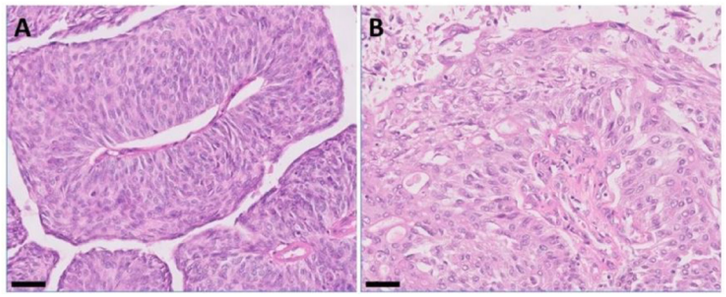


Figure 1: H&E histopathology images from nephroureterectomy. (A) Low-grade UTUC with characteristic low-grade features: organized and cohesive papillary architecture of monomorphic cells with preserved polarity. (B) High-grade UTUC with characteristic high-grade features: loss of organization, cohesiveness, and polarity of polymorphic cells.

The histopathologic grade and the stage of tumour invasion are essential for diagnosis, prognosis and treatment selection.[1] The oncologic effectiveness of kidney-sparing laser vaporization during ureterorenoscopy, for instance, can only be guaranteed in patients with low-grade and low-volume disease.[5] High-grade UTUC is generally treated by radical surgical resection (nephroureterectomy with bladder cuff excision or segmental ureterectomy). As a results, risk-stratification for adequate treatment selection of UTUC has become a key step in the diagnostic pathway.[1,6] The tumour volume may be assessed

with radiologic imaging while tumour grading requires the assessment of the tissue microarchitecture. The tissue is acquired by endoscopic biopsies during diagnostic ureterorenoscopies under general anaesthesia. However, the current diagnostic pathway lacks the ability to provide real-time histopathologic assessment for treatment selection during the diagnostic ureterorenoscopy. Accurate intraoperative risk-stratification could avoid a subsequent therapeutic ureterorenoscopy or could justify immediate laser ablation during the diagnostic ureterorenoscopy without histopathologic certitude from tissue biopsies. Moreover, intraoperative assessment alongside the current diagnostic approach could augment the diagnostic yield for UTUC grading as biopsies are often non-diagnostic.[7–11]

In the quest to fill this gap in the diagnostic pathway, in-vivo studies on probe-based optical imaging techniques, such as confocal laser endomicroscopy and optical coherence tomography (OCT), have provided promising results.[12–15] A previous study has suggested that visual assessment of OCT images allows for UTUC staging while quantitative assessment of the OCT signal enables UTUC grading.[15] The quantitative assessment is based on the attenuation coefficient (μ_{OCT}), which parameterizes the exponential decay of OCT signal with tissue depth. The μ_{OCT} depends on tissue-specific optical properties that arise from the nuclear and cellular organization and morphology.[16,17] As high-grade urothelial carcinoma has a tendency towards a more extreme nuclear and cellular disorganization compared to low-grade urothelial carcinoma, it is expected that the μ_{OCT} has a discriminating power to differentiate between low-grade and high-grade UTUC.

Indeed, Bus et al demonstrated that the μ_{OCT}, based on the assessment of the circular OCT grey-scale images, has the potential to be used as a cut-off for UTUC grading.[15] While this method would make it possible to analyse OCT images directly during ureterorenoscopy, it does not accurately account for the sensitivity roll-off and confocal point spread function. Therefore, the determined μ_{OCT} will be underestimated.

To correct for this underestimation, we developed software in which the circular OCT images were directly coupled to the raw amplitude OCT data. As a result, μ_{OCT} analysis based on the raw amplitude OCT data, corrected for the sensitivity roll-off and confocal point spread function, was possible based on selected regions of interest in the circular images. Furthermore, in the previous study the classification of low- and high-grade lesions on μ_{OCT} was based on the histopathologic grade from resection specimens only, which may have induced selection bias. In this study, we therefore also included patients in whom only a biopsy was taken, thereby increasing the number of low-grade lesions of low

tumour volume. The objective of this post-hoc analysis is to define the μ_{OCT} cut-off for grading of papillary UTUC with the developed software in the largest cohort of UTUCs currently available that were imaged in vivo by OCT.

Methods

Data collection

For this post-hoc analysis, OCT data was obtained from the study by Bus et al.[15] The study was approved by the institutional review board and was registered on Clinicaltrials.gov (NCT02326909). Written informed consent was obtained from all participants. Their study design was in line with the IDEAL stage 2b recommendations for explorative studies to evaluate the diagnostic accuracy of OCT for staging and grading of UTUC.[18] patients were eligible for the post-hoc analysis if ureterorenoscopic OCT images of papillary UTUC and histopathologic grading from either biopsies or radical resection specimens were available. Histopathology from resection specimen was not available in all patients because some received kidney sparing ureterorenoscopic laser ablation.

OCT imaging

OCT imaging was performed during ureterorenoscopy with the commercially available 1300 nm C7-XR™ OCT system (St. Jude Medical, Minnesota, USA) interfaced to a 2.7 Fr single-use C7 Dragonfly™ imaging probe. The OCT probe was introduced through the working channel of standard semi-rigid or flexible ureterorenoscopes (Karl Storz semi-rigid 9.5Fr, Karl Storz Flex XC, Olympus URF-V). As shown in Video 2, the sideward-looking OCT probe, linked to a rotary junction with pull-back system, scans a 54 mm long trajectory to create a 540-frame helical dataset of raw OCT amplitude data. The measurements are stored in two different multi-frame TIFF formats. The unprocessed linear data is stored as unsigned 16-bit integers. Each column in each frame represents a single A-scan (reflectivity versus tissue depth, i.e. amplitude scan). For visualization purposes, the amplitude data is log-transformed and resampled onto a polar coordinate system, resulting in circular color-coded images. The circular images are stored in unsigned 8-bit integer format. The lateral and axial resolution of the OCT system are 20 µm and approximately 10 µm, respectively, with a maximum imaging range of 7.5 mm.

Attenuation coefficient analysis

The μ_{OCT} analysis with the in-house developed software (LabVIEW 17.0) was performed by two OCT-users (J.E.F, D.M.B.), who were blinded for clinical information. The users were trained with a training set (J.E.F.) or had prior experience from the study by Bus et al (D.M.B.).[15] In the developed software,

circular and linear scans are coupled on a frame-to-frame basis (e.g. a single index of both multi-frame formats yields the same scan in circular and linear representation). An example of the software interface is given in Figure 2. The first A-scan in a linear image (right panel of Figure 2A) appears at 3 o'clock in the circular image (left panel of Figure 2A); subsequent A-scans follow in a clock-wise fashion.

The region of interest (ROI) for μ_{OCT} fitting was visually determined by identifying the papillary tumour in the circular OCT image (left panel Figure 2A). The selected ROI was then automatically highlighted in the linear scan (right panel Figure 2A). In the linear scan, the ROI was manually adjusted so that the largest area from the largest number of A-lines of homogenously appearing tumour was selected. Subsequently, the amplitude of the OCT signal in the selected OCT frame, averaged over the selected A-scans versus tissue depth, was used for the automated calculation of the μ_{OCT} (Figure 2B).

The μ_{OCT} was determined by fitting a single-backscattering OCT model to the averaged A-scan, considering additional signal decay due to instrumental factors. The average OCT amplitude is given by:

$$\langle A(z) \rangle = t(z) \cdot h(z) \cdot A \cdot exp(-\mu_{OCT} z) + noise$$

In the non-linear least squares curve fit, the amplitude A and attenuation coefficient μ_{OCT} were the free running parameters. The noise level is fixed at the average OCT amplitude in the noise region of the averaged A-scan.

The factor t(z) is the confocal point spread function which is parameterized by the effective Rayleigh length ZR and the position of the focus zf.[19]

$$t(z) = \left(\left(\frac{z - zf}{2Z_R} \right)^2 + 1 \right)^{-\frac{1}{2}}$$

The factor h(z) describes the sensitivity roll-off present in spectral domain OCT systems.[20] It is determined by the finite spectral resolution of such system:

$$h(z) = sinc\left(\frac{\pi}{2} \cdot \frac{z}{z_{max}} \right) \cdot exp\left(-\frac{\pi^2 \cdot s^2}{16 \cdot \ln(2)} \cdot \left(\frac{z}{z_{max}} \right)^2 \right)$$

Here, zmax is the maximum ranging depth of the OCT system (7.50 mm) and parameter s is the ratio of sampling resolution over spectral resolution.

The values of ZR, zf and s determine the system-induced decay of the OCT signal with depth. For accurate fitting of the μ_{OCT}, these values must be known. Based on the calibration measurements performed by Muller et al on Intralipid dilutions using five different Dragonfly probes, median values of ZR (0.67 mm), zf (1.95 mm) and s (1.29) could be determined and integrated as fixed parameters in the μ_{OCT} analysis software.[21]

μ_{OCT} analysis was repeated for a total of five OCT frames to reduce the possible effect of speckle and tissue heterogeneity. The frames were chosen as consecutive frames before and/or after the initially selected ROI in the same tumour. The number of five frames was chosen as a trade-off between speckle reduction and tumour size. For the final output, the average μ_{OCT} of these five measurements was multiplied by a factor of 1.4 to correct for the refractive index of water and tissue.

Histopathologic assessment

Biopsies were taken after OCT measurement during diagnostic ureterorenoscopy, using cup biopsy forceps (Piranha®, 3Fr Boston Scientific) or basket biopsy instrument (ZeroTip® 1.9Fr nitinol basket, Boston Scientific). The histopathologic assessment of ureterorenoscopic biopsies and resection specimens was performed by the department of pathology according to the standard clinical protocol and the WHO 2004 classification.[2] In the absence of histopathology from radical resections, the histopathologic grade from biopsies was considered as the reference standard. In cases where the histopathologic grade from biopsies was discordant with the grade from the resection specimen, the grade of the resection specimen was used as the reference standard.

Statistical analysis

Descriptive statistics were used to analyse patient and tumour characteristics. Mann-Whitney U testing was performed to assess the difference of the μ_{OCT} between low-grade and high-grade UTUC. The diagnostic accuracy of the μ_{OCT} cut-off values was analysed with a receiver operating characteristic (ROC) curve. The significance level of the area under the curve (AUC) was based on the comparison with the null hypothesis (AUC = 0.5). Statistical analyses were performed using SPSS (SPSS, Chicago, IL) and MedCalc V18.6 (MedCalc, Ostend, Belgium).

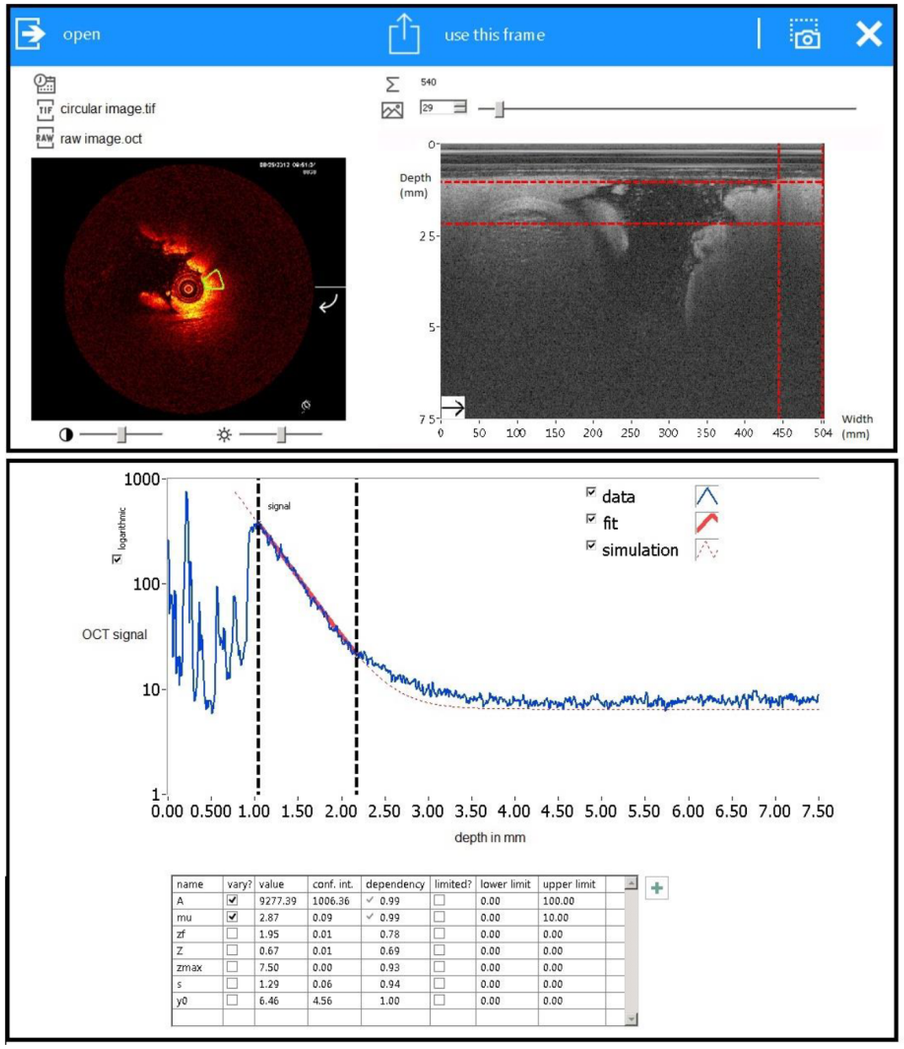


Figure 2: Screenshot of the in-house developed software interface for μ_{OCT} analysis; left panel 2A) manual selection of the region of interest from the circular OCT scan (marked by green outline); right panel 2A) coupled raw amplitude data of the linear scan with the stippled red square indicating the automated selection of the manually selected region of interest from the circular scan; B) determining the μ_{OCT} by fitting a single-backscattering OCT model to the averaged A-scans of the selected region of interest (in between stippled black lines).

Results

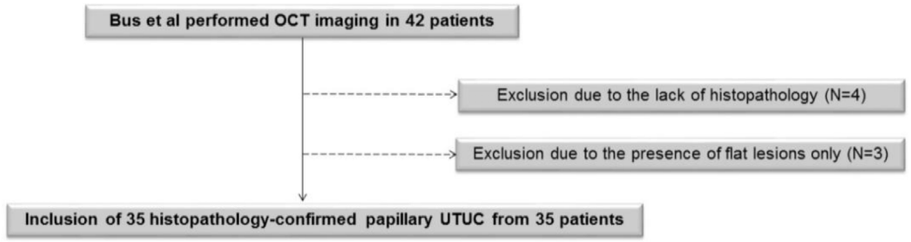
In total, 35 papillary UTUC ($n_{low\text{-}grade}$ = 17, $n_{high\text{-}grade}$ = 18) from 35 patients were included for data analysis (Figure 3). The patient and tumour characteristics are presented in table 1. Seven patients from the primary cohort were excluded for data analysis due to the lack of conclusive histopathology or the presence of carcinoma in situ only.

Table 1: Patient and tumour characteristics.

Age in years, mean (sd)		66 (13)
Gender, n (ration in %)	female / male	10 (29) / 25 (71)
Laterality of disease, n (ratio in %)	left / right	17 (49) / 18 (51)
Tumour location, n (%)	ureter / pyelocaliceal system	18 (51) / 17 (49)
Tumour size in mm, median (range)		25 (4 – 90)
Biopsy taken, n (% of total cohort)		32 (91)
Non-diagnostic result from biopsy, n (% of biopsies)		3 (9)
Histopathologic grade from biopsy, WHO 2004, n (ratio in %)	low-grade / high-grade	18 (62) / 11 (38)
Surgical resection performed, n (% of total cohort)		25 (71)
Type of surgical resection, n (ratio in %)	RNU / SU	22 (88) / 3 (12)
Histopathologic grade from resection, WHO 2004, n (ratio in %)	low-grade / high-grade	10 (40) / 15 (60)
Concordance of grade from biopsy with resection, n (ratio in %)	agreement / disagreement	16 (84) / 3 (16)

Table legends: RNU = radical nephroureterectomy, sd = standard deviation, SU = segmental ureterectomy

Figure 3: Flow chart of patient inclusion.



Ureterorenoscopic biopsies were taken in 32 patients. Surgical resections were performed in 25 patients. Twenty-two patients received both biopsies and surgical resections, in whom three biopsies were non-diagnostic (9% non-diagnostic yield of biopsies) and three biopsies were under-graded in comparison to the resection specimen.

The μ_{OCT} analysis was feasible in all 35 UTUC cases, meaning that a diagnostic yield of 100% was obtained. Representative circular OCT images and histopathology H&E staining of low grade and high grade papillary UTUC, as well as of normal upper tract urothelium are presented in Figure 4.

The median μ_{OCT} was 3.3 mm^{-1} (IQR 2.7 – 3.7 mm^{-1}) for low-grade UTUC and 4.9 mm^{-1} (IQR 4.3 – 6.1 mm^{-1}) for high-grade UTUC, as illustrated in Figure 5. The median μ_{OCT} values of these grade groups were statistically significant different (p=0.004).

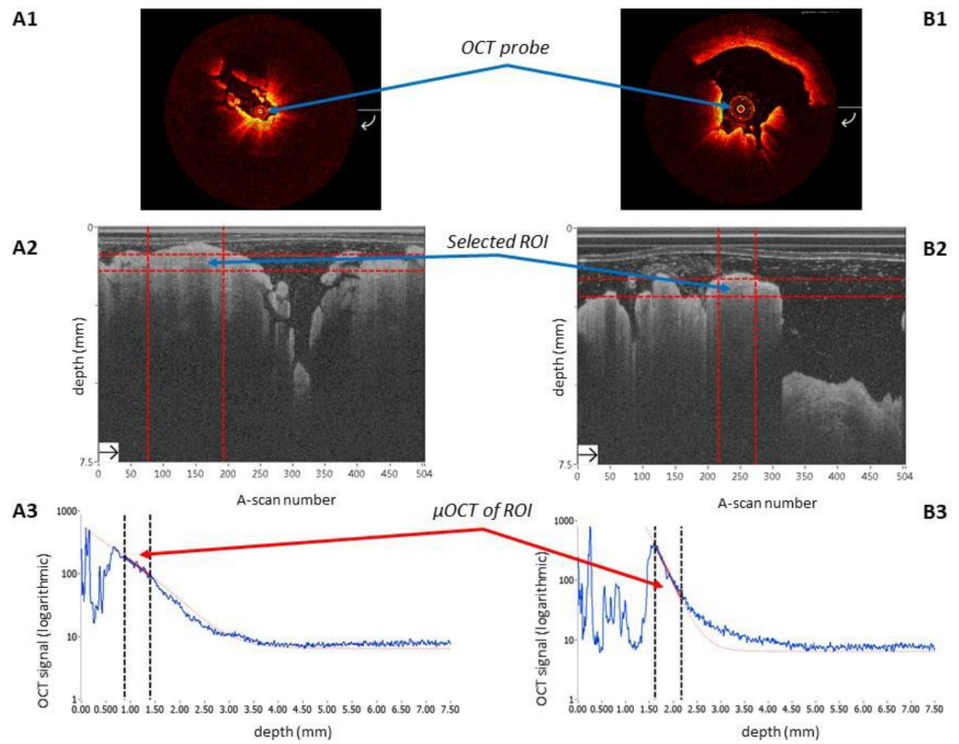


Figure 4: Left panel: circular OCT image of low-grade UTUC (A1) with the corresponding linear OCT image with the selected region of interest between the crosshairs (A2) and the corresponding average A-scan of the region of interest for μ_{OCT} analysis (A3). Right panel: circular OCT image of high-grade UTUC (B1) with the corresponding linear OCT image (B2) and the corresponding average A-scan of the region of interest for μ_{OCT} analysis (B3).
(Legends: μ_{OCT} = attenuation coefficient, derived from the gradient between the two vertical dotted black lines, OCT = optical coherence tomography, ROI = region of interest, UTUC = upper tract urothelial carcinoma).

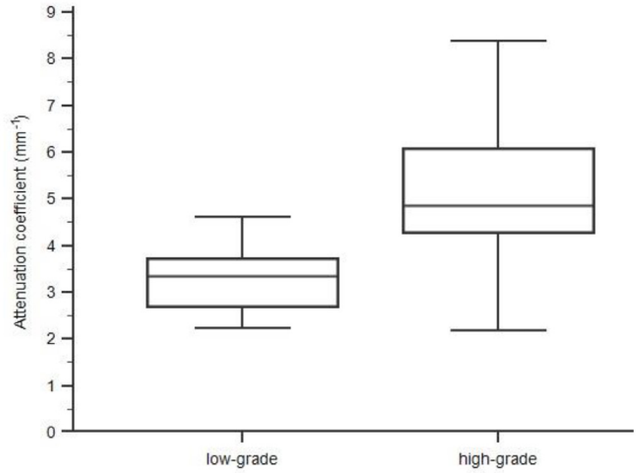


Figure 5: Box-and-whisker plots of the μ_{OCT} for the low-grade and high-grade UTUCs.
(Legends: The whiskers indicate the 95% confidence interval. The boxes represent the interquartile range.)

ROC analysis for the μ_{OCT} of low-grade versus high-grade UTUC yielded an AUC of 0.85 (95% CI 0.69 – 0.95, p<0.001), as presented in Figure 6. For this graph, a μ_{OCT} value of >4.0 mm^{-1} was identified as the optimal cut-off to discriminate high-grade from low-grade UTUC. This cut-off yielded a sensitivity of 83% and a specificity of 94%. In other words, the μ_{OCT} cut-off of >4.0 mm^{-1} correctly identified 15 of 18 high-grade UTUC with only 1 false positive of 17 low-grade UTUCs.

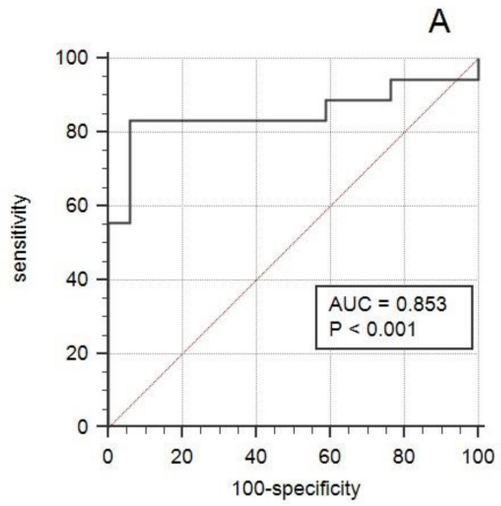


Figure 6: Receiver operating characteristic curve for the μ_{OCT} of all 35 low-grade and high-grade UTUC.

Discussion

In this study, we investigated a μ_{OCT} cut-off value for quantitative differentiation of low-grade and high-grade papillary UTUC. Our main finding is that a μ_{OCT} cut-off value of 4 mm^{-1} yields a high diagnostic accuracy to identify high-grade papillary UTUC. Implementation of the newly developed software for μ_{OCT} analysis at the point of care could lead to accurate intra-operative grading and hence immediate risk-stratification of UTUC during the diagnostic ureterorenoscopy. The results of this study should encourage future studies to validate the proposed cut-off value.[22]

In the study by Bus et al, the combined effect of the confocal point spread function and sensitivity roll-off were treated as a single offset (μ_{CAL}) to the attenuation coefficient. The μ_{OCT} value was then determined by adding μ_{CAL} to the fitted decay constant. This approach assumes a fixed value of the system induced attenuation and can be calibrated on samples with known μ_{OCT} as described by Almasian et al.[23] Critically, it is assumed that the focus is located at the position where the probe is in contact with the tissue boundary. For Dragonfly probes, however, this assumption is invalid. The apparent Rayleigh length ZR, focus position zf and roll-off parameter s were therefore calibrated independently and used as fixed parameters in the μ_{OCT} analysis of the present study. Furthermore, by extracting the data from the circular OCT images, Bus et al needed an additional adjustment for the log-transformation applied to these images for the μ_{OCT} analysis. In contrast, the present analysis uses the linear raw data directly as input to the fit. Owing to the improved definitions for the sensitivity roll-off and confocal point spread function in the newly developed software, the μ_{OCT} values for low-grade and high-grade UTUC were substantially higher in the present study. The corresponding sensitivity (83%) and specificity (94%) were similar to the sensitivity (87%) and specificity (90%) of the study by Bus et al.[15] Yet, as the accuracy of the present software was tested during the development phase in a validation set of intralipid dilutions, yielding similar values as published by Muller et al,[21] one may conclude that the μ_{OCT} values of the current study are more accurate than previously reported.[15]

Besides technical improvements, the sample size in the present study was substantially larger than in the previous study that proposed a μ_{OCT} cut-off value.[15] Increasing the sample size of this post-hoc analysis was achieved by additional inclusion of patients in whom only the histopathologic grade from ureterorenoscopic biopsies was available for comparison. In theory, the histopathologic assessment of resection specimens provides the most accurate histopathologic diagnosis, because the amount of tissue and structural integrity is maximized.[7] Yet, only relying on resection specimens may intro-

duce selection bias because resection is not the first choice of treatment in low-volume low-grade UTUC.[1] Moreover, due to grade heterogeneity within and in spatially separated regions, the histopathological grade of the complete resection specimen may not be representative for the region sampled by the local μ_{OCT} measurement.[24,25] Likewise, using the histopathologic grade from ureterorenoscopic biopsies as reference standard without precise co-localization with the region sampled by the local μ_{OCT} measurement may also lead to inaccurate comparison. Nevertheless, ureterorenoscopic biopsies should theoretically enable superior co-localization with the OCT measurement to rule out grade heterogeneity in spatially separated regions.

Due to the post-hoc character of the present study, however, co-localization of local μ_{OCT} analysis with the region biopsied was limited. Co-localization could only be based on reports from the time of ureterorenoscopy. The limitation of co-localization with regard to tumoral grade heterogeneity is illustrated by three cases of resected high-grade UTUC with μ_{OCT} values in the highest range but with low-grade UTUC in the preceding biopsies. In future, macroscopic co-localization should be assisted with the endoscopic images form ureterorenoscopy when using ureterorenoscopic biopsies as reference standard. Furthermore, the inclusion of cases with biopsies as reference standard balanced the prevalence of low-grade and high-grade UTUCs in this cohort, resulting in a better resemblance of target group. Nonetheless, macroscopic co-localization of the region assessed by the index test with the current reference tests cannot pre-empt a possible inaccuracy caused by intratumoral grade heterogeneity.[24,25] In this regard, it is interesting to note that the newly developed software for μ_{OCT} analysis can be implemented at the point of care, allowing for UTUC grading of the complete tumour volume during ureterorenoscopy. Evaluation of the complete tumour is clinically essential to rule out undergrading in case of grade heterogeneity. As reported for prostates and arteries, automated algorithms for ROI and tissue boundary detection are required to enhance real-time μ_{OCT} analysis and assessment of complete tumours.[26,27] Automatic boundary detection, however, might be difficult in papillary formations with varying distances between the OCT probe and the tissue edge.

For future studies, the highest certitude of histopathologic correlation for the assessment of an index test may be achieved in cases of concordant histopathologic grades from ureterorenoscopic biopsies and resection specimens. For the present study, for example, ROC analysis for this subgroup ($n_{low\text{-}grade}$ = 8, $n_{high\text{-}grade}$ = 8) resulted in a μ_{OCT} cut-off value of >3.92 mm^{-1} with a sensitivity of 100% and a specificity of 88% (Figure 7, AUC = 0.922, 95% CI 0.68 – 1.00, p<0.001). In contrast, the ROC analysis for the complete cohort did not reach a sensitivity of 100%. This limitation was caused by a false negative μ_{OCT} in

a high-grade lesion where extended tumour necrosis was explicitly described by the uropathologist. Necrosis-induced changes of the tissue-specific optical properties may result in a substantial reduction of the μ_{OCT}, and hence an underestimation of the µOCT for high-grade UTUCs.[28]

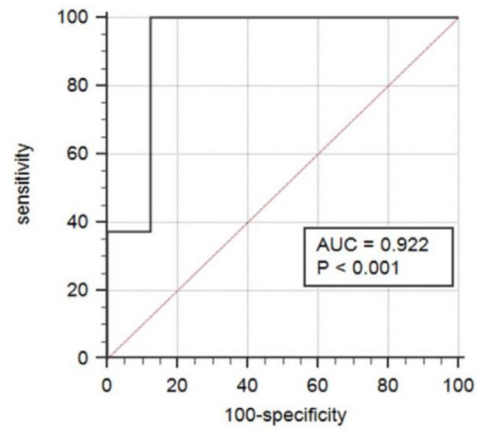


Figure 7: Receiver operating characteristic curve for the μ_{OCT} of the 16 cases with concordant histopathologic grades from biopsy and resection.

Relying on histopathology reports from different uropathologists may have introduced inter-rater variability due to the lack of distinct cut-off values for histopathologic grading. However, by using the WHO classification of 2004/2016, the inter-rater variability may have been minimized compared to assessment according to the WHO classification of 1973.[3] In future, a group of uropathologists should assess the histopathologic grade jointly to rule out intra- and inter-rater variability.

A technical limitation of μ_{OCT} analysis is the infeasibility of attenuation quantification in flat lesions, because of their marginal thickness.[15] Similarly, μ_{OCT} analysis might be hampered in papillary tumours of marginal tumour size, because a restriction of the lateral data increases the influence of speckle (contrast is reduced by the square root of the number of averages) on the raw OCT amplitude data. Increasing the number of averaged A-scans reduces the detrimental effect of speckle but is only feasible within tissue of sufficient homogeneity. Likewise, a small number of axial data points reduces the accuracy of the μ_{OCT} fit. Maximizing the depth of the fit is beneficial as long as the tissue within this range is homogeneous. Please note that the diagnostic yield of ureterorenoscopic biopsies was also limited in this study.

Besides quantitative assessment of the μ_{OCT}, other methods for quantitative OCT signal assessment might also facilitate tumour grading. Texture

analysis and integral depolarization factor analysis have also been reported for the differentiation of urothelial carcinoma.[29,30] In non-urologic diseases, similar approaches have been coupled to machine learning models with promising results.[31,32] Furthermore, technical alterations of the OCT system may provide further possibilities for quantitative image assessment.[33]

Conclusions

In the quest of optical biopsies for intra-operative risk-stratification of UTUC, this study proposes a μ_{OCT} cut-off value of 4.0 mm^{-1} for quantitative grade differentiation in ureterorenoscopic OCT images. The promising diagnostic accuracy calculations justify further studies to validate the proposed cut-off. Implementation of the software for the μ_{OCT} analysis in the current OCT systems may allow for ureterorenoscopic μ_{OCT} assessment at real time to enable for intra-operative risk-stratification of UTUC.

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List of abbreviations

CIS – carcinoma in situ
CLE – confocal laser endomicroscopy
RNU – radical nephroureterectomy
SU – segmental ureter resection
TURB – transurethral resection of bladder tumours
URS – ureterorenoscopy
UTUC – upper tract urothelial carcinoma
WHO – World Health Organization
WLC – white light cystoscopy

Chapter 6

Confocal Laser Endomicroscopy for the Diagnosis of Urothelial Carcinoma in the Bladder and the Upper Urinary Tract: Protocols for two Prospective Explorative Studies

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M.L. Liem*, J.E. Freund*, J. Baard, D.M. de Bruin,
M.P. Laguna Pes, C.D. Savci-Heijink, T.G. van Leeuwen,
T.M. de Reijke, J.J.M.C.H. de la Rosette
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**Both authors contributed equally.*

A peer-reviewed video titled 'Confocal Laser Endomicroscopy for the Diagnosis of Urothelial Carcinoma in the Bladder and the Upper Urinary Tract' was published alongside:

J.E.Freund*, E.I.M.L. Liem*, J. Baard, G.M. Kamphuis, M.P. Laguna,
T.M. de Reijke, J.J.C.M.H. de la Rosette, D.M. de Bruin.
Videourology. 2018;32:4.

**Both authors contributed equally.*

Abstract

Background: Visual confirmation of a suspicious lesion in the urinary tract is a major corner stone in diagnosing urothelial carcinoma. However, during cystoscopy (for bladder tumours) and ureterorenoscopy (for tumours of the upper urinary tract) no real-time histopathologic information can be obtained. Confocal laser endomicroscopy (CLE) is an optical imaging technique that allows for in vivo high-resolution imaging and may allow real-time tumour grading of urothelial lesions.

Objective: The primary objective of both studies is to develop descriptive criteria for in vivo CLE images of urothelial carcinoma (low-grade, high-grade, carcinoma in situ) and normal urothelium by comparing CLE images with corresponding histopathology.

Methods: In these two prospective clinical trials, CLE imaging will be performed of suspicious lesions and normal tissue in the urinary tract during surgery, prior to resection or biopsy. In the bladder study, CLE will be performed in 60 patients using the Cystoflex UHD-R probe. In the upper urinary tract study, CLE will be performed in 25 patients during ureterorenoscopy, who will undergo radical treatment (nephroureterectomy or segmental ureter resection) thereafter. All CLE images will be analysed frame by frame by three independent, blinded observers. Histopathology and CLE-based diagnosis of the lesions will be evaluated. Both studies comply with the IDEAL stage 2b recommendations.

Results: Presently, recruitment of patients is ongoing in both studies. Results and outcomes are expected in 2018.

Conclusions: For development of CLE-based diagnosis of urothelial carcinoma in the bladder and the upper urinary tract, a structured conduct of research is required. This study will provide more insight in tissue-specific CLE criteria for real-time tumour grading of urothelial carcinoma.

Trial Registration: Confocal Laser Endomicroscopy: ClinicalTrials.gov NCT03013894; and Dutch Central Committee on Research Involving Human Subjects NL55537.018.15. Confocal Laser Endomicroscopy in the upper urinary tract: ClinicalTrials.gov NCT03013920; and Dutch Central Committee on Research Involving Human Subjects NL52989.018.16.

Introduction

Urothelial carcinoma is the most common malignancy of the urinary tract. The majority of these tumours is located in the bladder and only 5% are located in the upper urinary tract.[1] For bladder cancer, direct visualization of the urothelium with white light cystoscopy (WLC) is the cornerstone for diagnosis and follow-up. Despite its effectiveness and established role, WLC has limitations, such as its diagnostic accuracy, especially for carcinoma in situ (CIS).[2] Histopathologic grading and staging of urothelial carcinoma are essential for diagnosis, prognosis and choice of therapy. However, real-time histopathologic assessment is lacking during cystoscopy in an outpatient setting as well as in the operating theatre. For upper urinary tract urothelial carcinoma (UTUC), ureterorenoscopy (URS) with endoscopic biopsies of suspicious areas is considered the diagnostic standard. Also for UTUC, histopathologic evaluation is essential for prognosis and treatment selection as endoscopic treatment is reserved for low grade tumours only.[3] Similarly to the diagnostics of bladder cancer, real-time histopathologic assessment is lacking during URS. The use of optical imaging techniques, such as Confocal Laser Endomicroscopy (CLE) may enable real-time optical biopsies to overcome these limitations for bladder cancer and UTUC evaluation.

CLE is a fiber optic probe-based imaging technique that enables real-time in-vivo optical sectioning of tissue. The Cellvizio®CLE system uses a low-energy 488 nm laser source. The presence of a pinhole limits the detection to in-focus backscattered fluorescent light, enabling high resolution imaging in a single horizontal plane. CLE imaging requires the administration of a fluorescent contrast agent. The most commonly used fluorescent dye is fluorescein. Topical or intravenous application of fluorescein stains the extracellular matrix and enables visualization of the cellular microarchitecture and morphology. Tissue types can be differentiated based on these specific cellular features. For in vivo endoscopic CLE imaging, various sized probes with different image properties are commercially available. CLE was initially applied for in vivo imaging in the gastrointestinal tract, and applications in pulmonology are explored.[4–6] In urology, CLE was first examined in the bladder. It seemed feasible to differentiate between normal mucosa and urothelial carcinoma using CLE imaging in a pilot study. Based on histopathology from resected bladder tumours, CLE criteria to differentiate between normal bladder tissue, low-grade and high-grade bladder tumours were proposed.[7,8] These criteria have also been suggested for the upper urinary tract as the histologic morphology and microarchitecture are alike. However, a CLE probe with different imaging properties is used in the upper urinary tract and only small patient cohorts have been evaluated.[9,10]

The development of CLE towards real-time optical biopsies of urothelial carcinoma may lead to advances in diagnosis and prognosis and may affect the cost-benefit of the disease management. Currently, bladder cancer is the most expensive solid malignancy per patient. The high recurrence rate of early stage tumours with long-term survival and adjuvant treatments with close follow-up results in high costs.[11,12] Even though laser fulguration of low-risk tumours has been performed in outpatient settings, it is not widely used due to the lack of histologic confirmation and therewith the risk of inadequate treatment.[13,14] Immediate evaluation of tumour grade with CLE could potentially increase the application of laser fulguration and enable treatment of real-time confirmed low-grade tumours in an outpatient setting. Application of laser fulguration in an outpatient setting would lead to an increase in availability of treatment of low-grade bladder cancer and reduction of medical costs. Potentially, CLE may also allow for real-time evaluation of surgical radicality and therewith reduce recurrence rates in urothelial cancer. In the upper urinary tract, CLE has the potential to improve the diagnostic approach for UTUC.

Accurate staging and grading of UTUC remains challenging. Visual white light assessment of UTUC grade during URS is inaccurate in approximately 30% of the cases.[15] Moreover, the restricted anatomic space of the upper urinary tract and the subsequent miniaturization of tissue harvesting instruments limit the yield of ureteroscopic biopsies. The diagnostic yield and the diagnostic accuracy for tumour stage of endoscopic biopsies are limited.[16,17] However, tumour grade from endoscopic biopsies may indicate tumour stage.[18,19] As such, tumour grade from endoscopic biopsies is a major decisive factor for endoscopic treatment eligibility. Though, in 69-90% of endoscopic biopsies, tumour grade is in concordance with the histopathologic grade from radical resection.[17,18,20,21] Moreover, endoscopic biopsies hold a risk of complications.

CLE may overcome such diagnostic limitations for tumour grade assessment. Optical real-time assessment of tissue type and tumour grade could aid perioperative clinical decision-making. Histologic assessment without tissue biopsies could prevent possible impaired endoscopic vision after biopsies during URS. Additionally, the digital data from CLE imaging allows for real-time computer aided diagnosis with software, augmenting the practical and diagnostic value of optical imaging techniques. Further exploration of different optical imaging modalities for tumour diagnosis may lead to multimodal optical biopsies for real-time tumour grading and staging, possibly replacing tissue biopsies in future. However, a structured conduct of research is required to guide us

towards optical biopsies. The aim of these two study-protocols is to contribute to the development of essential knowledge for CLE based diagnosis of urothelial carcinoma in the bladder and the upper urinary tract. In this paper, we describe two study protocols for CLE in the urinary tract together as both protocols share many methodological and disease-specific similarities.

Methods

Study objectives

The primary objective of both studies is to develop descriptive criteria for in vivo CLE images of urothelial carcinoma (low-grade, high-grade, CIS) and normal urothelium by comparing CLE images with corresponding histopathology.

Secondary objectives are to develop a CLE image atlas of the urinary tract, to assess the technical feasibility and procedure related adverse events, to assess CLE image quality, to qualitatively evaluate CLE images, to preliminarily assess the diagnostic yield, and to establish an estimation of the diagnostic accuracy of CLE based diagnosis in comparison with histopathology and to assess inter-observer agreement.

Study design

Approval of the local medical ethical committee was obtained for both study protocols (registry number: NL55537.018.15 and NL52989.018.16). Both studies are prospective, single centre, in vivo, observational human studies to assess CLE features of normal urothelium and urothelial carcinoma (low-grade, high-grade or CIS) in the bladder and in the upper urinary tract. Both explorative studies are in agreement with the IDEAL stage 2b recommendations.[22] The two study protocols differ mainly in the location of urothelial carcinoma, and subsequently the surgical approach, the type of CLE probe, the administration of fluorescein and the reference standard. Differences in protocols are explained separately and listed in table 1.

Table 1: Differences between the two study protocols.

	CLE bladder study *(NL55537.018.15)*	**CLE upper urinary tract study *(NL52989.018.16)***
Population	60 consecutive patients with primary or recurrent bladder tumour	25 patients with UTUC that will undergo a RNU or SU after diagnostic URS with CLE imaging
Inclusion criteria	- Bladder tumour or possible CIS - Scheduled for TURB - Signed informed consent	- Suspicion of UTUC - Scheduled for diagnostic URS - Signed informed consent
Exclusion criteria	- Allergy for fluorescein - Possible pregnancy or lactating women	- Allergy for fluorescein - Possible pregnancy or lactating women - Patients not eligible for RNU
Urologic instruments at use	Karl Storz 22 Fr cystoscope with 0° optics for CLE imaging, and Karl Storz or Olympus 26 Fr resectoscope for transurethral resection	Karl Storz Flex Xc or Olympus V2 8.5 Fr flexible digital ureterorenoscope
Contrast agent	Topical application of 300-400 mL of 0.1% fluorescein via Foley catheter and left indwelling for 5 minutes	Topical application of 0.5 mL 2.5% fluorescein via working channel for immediate imaging
CLE probe	Cystoflex UHD-R - Diameter 2.6 mm - Lateral resolution 1 µm - Field of view 240 µm - Imaging depth 50-65 µm	Uroflex-B - Diameter 0.85 mm - Lateral resolution 3.5 µm - Field of view 320 µm - Imaging depth 40-70 µm
Histopathologic reference standard	*En-bloc* resected bladder tumour	RNU or SU

For both studies, CLE images are recorded with a fiber optic probe-based system (Cellvizio® 100 series, Mauna Kea Technologies, Paris, France). The Cystoflex UHD-R probe of 8.4 Fr (Mauna Kea Technologies, Paris, France) is used for CLE imaging in the bladder. The Uroflex-B probe of 2.7 Fr (Mauna Kea Technologies, Paris, France) is used in the upper urinary tract. The smaller Uroflex-B probe contains less optical fibers and therewith a lower image resolution compared to the Cystoflex UHD-R probe. Both forward-looking probes are illustrated in table 1 and figure 1.

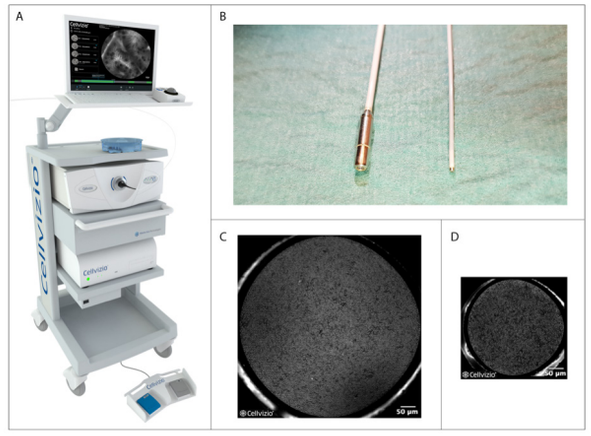

Figure 1: Cellvizio® CLE system and CLE probes. A) Cellvizio® CLE system. B) The two flexible probes used in the urinary tract. On the left the Cystoflex UHD-R probe for the bladder with a diameter of 2.6 mm. On the right the Uroflex-B probe for the upper urinary tract with a diameter of 0.85 mm. C) RAW image of the Cystoflex UHD-R probe displaying the single fibers. D) RAW image of the Uroflex-B probe displaying the single fibers.

CLE imaging requires the application of a fluorescent contrast agent. Fluorescein (fluoresceinedisodium, Fresenius Kabi, Zeist, The Netherlands) is a non-toxic and commonly used fluorescent dye for CLE imaging.[23] It stains the extracellular matrix and is administered topically prior to CLE imaging during the surgical procedure.

In both studies, patients will undergo in-vivo CLE imaging during surgery, prior to resection or biopsy of suspicious areas for standard histopathologic assessment. The probes are introduced through the working channel of the standard endoscopes. In the bladder, a Karl Storz cystoscope of 22 Fr with 0° optics is used for CLE imaging. Transurethral resection is subsequently performed with a Karl Storz or Olympus resectoscope of 26 Fr. For CLE imaging of the upper urinary tract, a flexible digital ureterorenoscope of 8.5 Fr is used (Karl Storz Flex Xc or Olympus V2). After placing the probe in direct contact with the region of interest, image sequences of 8–12 frames per second of the real-time visualization of the cellular microarchitecture are recorded (supplemental videos 1 and 2). In general, recording is conducted in both protocols twice for one minute of the region of interest. In case of multiple regions of interest, multiple CLE recordings are performed. At a later stage, recorded CLE images will be analysed independently by three blinded observers and compared to the corresponding histopathology. For CLE imaging in the bladder, the reference standard for comparison of histopathology will be the specimen of the en-bloc resected lesion. For the upper urinary tract, the reference standard will be the specimen of the radical resection (radical nephroureterectomy (RNU) or segmental

ureter resection (SU)). Histopathology analysis is performed according to the standard clinical protocol and is performed by a specialized uropathologist (C.D.S.H.), who is blinded for the CLE images. A follow-up of 30 days is considered to register any adverse events following the study procedure.

Population and sample size
Patients eligible for either study are >18 years with a suspicious lesion in the urinary tract, scheduled for either transurethral resection (TURB) (lower urinary tract) or diagnostic URS (upper urinary tract). The main exclusion criteria are fluorescein allergy and pregnancy (table 1). All patients will be recruited in the AMC Hospital (Amsterdam, The Netherlands), and all study procedures will be performed in this institution. Patients will be informed about the study in oral and written form. Patient inclusion is confirmed by signing written informed consent. Patients will only be included in one study at the time. A total of 60 consecutive evaluable patients with bladder tumours or suspicion of CIS will be included in the bladder cancer study. Based on prevalence, we expect to include 32 low-grade, 22 high-grade, and 6 CIS lesions. For the upper urinary tract study 25 patients with UTUC will be included that will undergo a radical treatment after the diagnostic URS. However, the indication for radical treatment is determined after diagnostic URS. In general, about one third of the UTUC patients are treated with radical surgery in our centre. Therefore, we expect to include 70 consecutive UTUC patients to reach the total number of 25 patients who will undergo radical treatment. Considering the possible selection bias for radical treatment, we expect to include 10 low-grade, 12 high-grade, and 3 CIS lesions. These sample sizes are based on prior publications and comply with the IDEAL recommendations for explorative studies.[7,9,22]

Study procedures
Protocol CLE in the bladder (NL55537.018.15)
In the operating theatre prior to the TURB under general or regional anaesthesia, visual inspection with WLC and image enhancement modalities (narrow band imaging or Image1S) will be performed. At least one suspicious lesion (papillary or flat) and one region of normal appearing bladder tissue will be selected for CLE imaging. The regions of interest will be marked laterally with the cautery electrode for topographic matching. After marking the regions of interest, 300-400 mL of fluorescein (0.1% fluorescein diluted in saline) will be administered intravesically using an indwelling catheter. After instillation of the fluorescein for 5 minutes, the bladder will then be emptied and excessive fluorescein will be rinsed out. The Cystoflex UHD-R probe will be introduced through the working channel of a 22 Fr Karl Storz cystoscope with 0o optics. After placing the probe into direct contact and perpendicular to the tissue, CLE images will be recorded twice for approximately 1 minute of each marked region. After CLE imaging, the tumour will be resected en-bloc and a small chip of the marked normal urothelium will be resected for histopathologic matching. Transurethral resection is performed with a Karl Storz or Olympus resectoscope of 26 Fr.

Protocol CLE in the upper urinary tract (NL52989.018.16)
The complete ureter and renal pelvis are inspected with WLC and image enhancement modalities (narrow band imaging or Image1S) during standard flexible digital ureterorenoscopy under general anaesthesia to identify regions of interest. Only in case of visually confirmed upper tract tumours during URS, study-related activities will be performed. If regions of interest are identified, the region that is most easily accessible for endoscopic biopsies is selected for CLE imaging. Fluorescein (0.5 mL of 2.5% fluorescein diluted in saline) is administered through the working channel. The Uroflex-B probe is introduced via the working-channel of the 8.5 Fr flexible digital ureteroscope (Karl Storz Flex Xc or Olympus V2) and placed into direct contact with the region of interest for immediate CLE imaging. Each region of interest is imaged twice for approximately 1 minute with CLE. After imaging, endoscopic biopsies for the standard diagnostic process are taken from the same location. Depending on the histopathologic diagnosis, the indication for a radical treatment will be determined.

Data analysis
The method of analysis is identical for both study protocols. Demographic and disease specific characteristics of the study populations (age, sex, medical history of urothelial carcinoma, tumour focality, tumour location and tumour size) will be collected. Three blinded observers, consisting of two researchers (E.L. & J.F.) and one uropathologist (C.D.S.H.), will independently analyse CLE images frame by frame in an offline setting with the Cellvizio Viewer software (Mauna Kea Technologies, Paris, France). Modified criteria for CLE image evaluation will be used for analysis (table 2).[8]

Table 2: Modified CLE image characteristics and their variables for analysis.

CLE feature	Variables
Papillary aspect	present | not present
Polarity of cells	present | not present
Organisation of cells	organized | disorganized
Cohesiveness of cells	cohesive | discohesive
Cellular morphology	monomorphic | pleiomorphic
Definition of cell borders	distinct | indistinct
Vasculature	capillary network | fibrovascular stalk | large vessels

The CLE image quality per region of interest will be scored on a Likert scale as poor, fair or good. Image quality will be used for qualitative evaluation of the technique and for subgroup analysis. Based on histologic features, for each region of interest a CLE based diagnosis will be made by each observer as benign urothelium or urothelial carcinoma. The diagnosis of urothelial carcinoma is subdivided in low-grade or high-grade urothelial carcinoma (WHO 2004 classification), CIS, or as inconclusive. An inconclusive CLE diagnosis is defined as poor image quality where CLE features cannot be assessed. After individual analysis, a consensus will be reached for the CLE based diagnosis by all three observers for each region of interest. The appropriateness of consensus of the CLE based diagnosis is evaluated by viewing the endoscopic images. After determining CLE based diagnosis and consensus, CLE images will be compared to the corresponding histopathology specimen (either en-bloc resected bladder tumour or RNU/SU specimen) for evaluation of the concordance of CLE based diagnosis with histopathologic diagnosis. Differences between diagnosed groups will be analysed with Chi-square test. For an initial evaluation of the diagnostic value, sensitivity and specificity will be calculated based on a 3x3 table where CIS is classified as high-grade tumour. Proportions of specific agreement and Fleiss Kappa analysis will be used for inter-observer agreement of CLE based diagnosis. A schematic overview of the data analysis is displayed in figure 2.

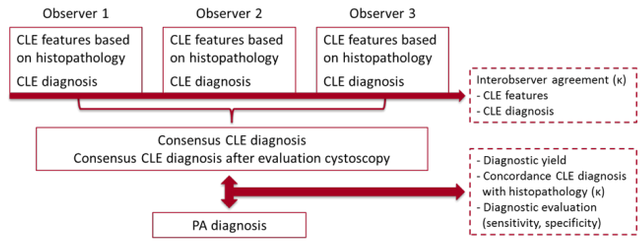

Figure 2: A schematic overview of the data analysis plan.

Safety

The investigators will monitor patient safety. They can withdraw a patient from the study for medical reasons. In accordance to section 10, subsection 4, of the "Wet Medisch-Wetenschappelijk Onderzoek met Mensen" (medical research involving human subjects act in The Netherlands), the investigators will suspend the study if there is sufficient ground that continuation of the study will jeopardise patient health or safety. The investigators will notify the accredited Institutional Review Board (IRB) if this is the case. In case of an adverse event or serious adverse event, the responsible authorities will be informed.

Risks and benefits

There are no direct benefits for patients participating in these two studies. In the future, however, the results of these studies may be important for patients diagnosed with a tumour in the urinary tract. All patients participating in both studies are scheduled for standard treatment of tumour(s) of the urinary tract; either TURB or URS with endoscopic biopsies. CLE is a minimally invasive imaging technique that can be performed in conjunction with conventional endoscopic treatment. Previous studies of CLE combined with topical administration of fluorescein have proven to be safe.[7,24] Fluorescein is a commonly used fluorescent dye, and its safety is well established for its use in ophthalmological angiography.[25] In patients not at risk for a previously demonstrated allergic reaction, this dye is safe. Patients with a known allergic reaction to fluorescein cannot participate in this study.

Discussion

CLE is an optical imaging technique that may enable real-time optical biopsies. The exploration of tissue specific CLE criteria is essential for the development towards real-time tumour grading of urothelial lesions. Both trials will provide more insight into CLE features of urothelial carcinoma in the bladder and the

upper urinary tract and into its diagnostic value.

The design of the bladder protocol aims for topographic matching of CLE images with the resected specimen. The cauterisation marks placed laterally to the region of interest prior to CLE imaging ensures that imaging and resection is done of the exact topographic tissue. Nonetheless, it will be challenging to create an identical histopathological slide of the resected specimen in exactly the same plane as the imaged tissue. We assume that this approach is the closest approximation for topographic matching without interference in the standard clinical histopathologic process.

The study design of the upper urinary tract protocol will lead to a surplus of study measurements, considering that only about one third of the UTUC patients will receive radical surgery as treatment. Since mainly patients with high-grade or high-volume low-grade tumour will qualify for radical treatment, selection bias could be a risk. The data acquired of patients who are not suitable for radical treatment enables secondary analysis for the comparison of CLE images with the histology of endoscopic biopsies of the imaged regions of interest. In the current study design identical topographic matching of CLE images in the upper urinary tract with the specimen of the RNU is limited by the fact that in general the diagnostic URS with CLE imaging is not performed during the same procedure as the radical resection. However, topographic matching is approximated by tumour mapping during URS (mapping and annotation of location, size and appearance) for identification of lesions during the histopathologic assessment.

As with all new techniques, a learning curve for the handling and image interpretation may be expected for CLE. Besides potential intra-observer variability, the learning process might also influence the CLE image quality of the first cases. We aim to limit the number of users to a minimum number of experienced endourologists to minimize the potential effect of a learning curve.

Despite these limitations, we expect that the results of these trials will contribute to determining the role of CLE imaging for the diagnosis of bladder cancer and UTUC in clinical practice. In the light of the limitations of cystoscopy and URS, CLE holds the potential to enable real time tumour grading of urothelial carcinoma.

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Supplements

Multimedia Appendix 1: A demonstration of CLE imaging of a bladder tumour. The CLE probe is in direct contact with the bladder tumour for CLE imaging; https://www.researchprotocols.org/api/download?filename=f526a3de05368e5699d-7a8ab03549920.mp4&alt_name=8862-143218-1-SP.mp4.

Multimedia Appendix 2: A demonstration of CLE imaging of an upper urinary tract tumour. The CLE probe is in direct contact with the tumour for CLE imaging; https://www.researchprotocols.org/api/download?filename=7e156e69f-2d0a741c2cad95c5d07a3c1.mp4&alt_name=8862-143219-1-SP.mp4.

Chapter 7

Confocal Laser Endomicroscopy for Upper Tract Urothelial Carcinoma: Validation of the Proposed Criteria and Proposal of a Scoring System for real-time Tumour Grading

J.E. Freund, E.I.M.L. Liem, C.D. Savci-Heijink, J. Baard, G.M. Kamphuis, J.J.M.C.H. de la Rosette, D.M. de Bruin
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List of Abbreviations

AUC – area under the curve
CI – confidence interval
CLE – confocal laser endomicroscopy
NPV – negative predictive value
PPV – positive predictive value
ROC – receiver operating characteristic
SD – standard deviation
UC – urothelial carcinoma
UCB – urothelial carcinoma of the bladder
UTUC – upper tract urothelial carcinoma

Abstract

Purpose: Confocal laser endomicroscopy (CLE) is a fluorescence-based fiber optic imaging technique with the potential for intraoperative grading of upper tract urothelial carcinoma (UTUC). This study aims to (1) investigate the prevalence of the previously proposed CLE criteria for bladder cancer in papillary UTUC, (2) estimate the diagnostic value of CLE for UTUC grading and (3) propose a scoring system for a more quantifiable approach of CLE-based grading of UTUC.

Materials and methods: Ureterorenoscopic CLE was performed in patients with UTUC. Following CLE imaging, co-localized biopsies were taken for histopathologic comparison. Postoperatively, two blinded raters assessed the CLE images.

Results: Fifty-three papillary UTUCs (34 low grade and 19 high grade) were imaged with CLE in 36 patients. All the previously described CLE criteria were identifiable in varying proportions. After excluding 10 non-diagnostic recordings (5 low grade and 5 high grade) due to insufficient image quality, the histopathologic grade was correctly identified with CLE in 26 low-grade UTUCs (90%) and in 12 high-grade UTUCs (86%). The most prevalent CLE criteria with the highest diagnostic potential were cellular organization, morphology and cohesiveness of cells. A scoring system was proposed with these criteria, which yielded similar diagnostic accuracies.

Conclusions: Based on the previously proposed criteria, CLE enables accurate grading of papillary UTUC at a non-diagnostic rate of 19%. The most prevalent CLE criteria with the highest diagnostic potential for grading of papillary UTUC are cellular organization, morphology and cohesiveness of cells. The proposed scoring system may simplify the assessment of CLE images for UTUC grading but external validation is required.

Introduction

The oncologic effectiveness of the kidney-sparing treatment for upper tract urothelial carcinoma (UTUC) can only be warranted in selected patients.[1,2] Risk-stratification of UTUC has, therefore, become an essential step in the diagnostic pathway.[3] Endoscopic laser ablation is the treatment of choice in low-risk UTUC, while radical surgical resection is indicated in high-risk cases.[2]

The histopathologic tumour grade is a decisive factor for the risk-stratification. Consequently, the need for grade identification has augmented the importance of ureterorenoscopic biopsies. Real-time intraoperative risk-stratification by histologic assessment is lacking in the current diagnostic work-up.

In approximately 10 to 40% of ureterorenoscopic biopsies, the histopathologic grade is discordant with the tumour grade from surgical resection specimens.[4–8] Additionally, the diagnostic yield of ureterorenoscopic biopsies for UTUC grading ranges from 80 to 90%.[4–6,8]

Confocal laser endomicroscopy (CLE) is a fluorescence-based fiber optic imaging technique that has been investigated for differentiation of urothelial carcinoma (UC). These investigations have resulted in the proposal of CLE criteria for UC grading in the bladder and the upper tract.[9–11] Despite promising feasibility studies in the upper tract, until now, the proposed CLE criteria have only been validated for urothelial carcinoma of the bladder (UCB).[12–14] With regard to the similarity in histology of UCB and UTUC, identical CLE criteria are anticipated.[15] However, in comparison to cystoscopic CLE imaging, a smaller CLE probe must be used for the upper urinary tract. We hypothesize that the reduced optical resolution, the smaller field of view and the larger depth of confocal plane of the smaller ureterorenoscopic CLE probe will influence the visual appearance and thus the prevalence of the proposed CLE criteria in UTUC.[16,17] As a result, validation of the proposed CLE criteria for UTUC is required.

The first objective of this study is to identify the prevalence of the proposed CLE criteria for UCB in papillary UTUC. Secondly, the diagnostic accuracy of CLE for UTUC grading, including inter-rater agreement analysis, is evaluated. Thirdly, based on the CLE criteria with the highest diagnostic potential, we aim to propose a CLE-based scoring system for a more quantifiable approach towards CLE-based grading of UTUC.

Materials and Methods

Study design

The study design was in line with the IDEAL stage 2b recommendations and approved by the institutional review board.[18] The study was registered at the Dutch Central Committee on Research involving Human Subjects (NL52989.018.16) and at Clinicaltrials.gov (NCT03013920). This prospective clinical trial was carried out as previously described and conducted according to the guidelines of good clinical practice.[16]

Patients

Adult patients, planned for diagnostic ureterorenoscopy (URS) due to the suspicion of UTUC or for follow-up after kidney sparing treatment in the Amsterdam University Medical Centers, location AMC, were eligible for this study. Exclusion criteria were fluorescein allergy and pregnancy. Written informed consent was obtained from all participants. After inclusion, patients could be disqualified for the study due to the absence of visible lesions during URS. Furthermore, tumours could be disqualified from the study due to local recurrence at the same location as imaged during prior study participation.

Study procedure

The study procedure was conducted as previously reported.[16] In short, if a suspect upper tract lesion was visualized during URS, CLE imaging of this lesion was performed. In case of multifocality, the best accessible lesion was imaged. The 2.7 Fr Uroflex-B probe, interfaced with the 488 nm laser system, was used for CLE imaging. These multifiber-based probes yield a field of view of 320 µm, a lateral resolution of 3.5 µm in a confocal plane from 40 to 70 µm imaging depth.

CLE imaging was performed by experienced endourologists who had previously used CLE for UCB imaging.[14] Via the ureteroscope's working channel, 0.5 mL of 2.5% fluorescein solution was injected onto the region of interest for CLE imaging. The Uroflex-B CLE probe was then introduced via the working channel of the semirigid or flexible ureteroscope and was placed in direct contact with the tissue of interest.[19] At least two CLE recordings of one minute (8–12 frames/second) were obtained per lesion. Subsequently, a ureterorenoscopic biopsy was taken from the imaged lesion. Histopathologic workup and analysis were performed according to standard clinical protocol by a uropathologist (CDS), blinded for CLE images. UTUCs were graded according to the WHO 2004 classification.[15] The histopathologic grade from the tissue biopsies was used as the reference test.

CLE image assessment

The presence of the proposed CLE criteria (papillary configuration, organization of cells, cohesiveness of cells, cellular morphology, definition of cell borders, vasculature and polarity) was assessed by two experienced CLE raters (JEF, CDS). Both raters were trained with a CLE training module and the assessment of CLE recordings of UCB.[10,14] After a washout time of at least 3 months after obtaining CLE recordings, both raters, blinded to any clinical information and histopathology, evaluated the CLE recordings individually offline with the Cellvizio® Viewer software (Mauna Kea Technologies, Paris, France). Based on the UCB CLE criteria, the observers graded the recordings as low-grade or high-grade UTUC. In case of insufficient image quality, the CLE recording was considered as non-diagnostic.

After individual assessment, consensus for the CLE criteria and the CLE-based grading was reached for each lesion. The analysis of the prevalence of CLE features and the comparison of the CLE-based grading with the histopathology of the biopsied tissue were performed with the results of the consensus.

Sample size and statistical analysis

The sample size was based on the IDEAL recommendations for explorative studies and is in line with previously published CLE studies on UCB.[11,14,18] Flat lesions were excluded for the final analysis.

For the first objective, descriptive statistics were used to analyze the prevalence of CLE features for UTUC grade.

For the second objective, the diagnostic accuracy was assessed by estimating the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each individual CLE criterion and overall CLE-based grading. These estimations were calculated in comparison with the histopathologic grade by 2x2 tables for the cohort with and without the non-diagnostic CLE yield. The non-diagnostic yield was defined as the proportion of lesions with non-diagnostic CLE recordings. The inter-rater agreement for the CLE criteria and CLE-based grading was determined as percentage agreement between the two raters. A threshold of minimally 80% agreement was considered as acceptable agreement.[20]

Thirdly, the CLE criteria with the highest prevalence, PPV and NPV and with both sensitivity and specificity of greater than 50% were selected for the proposal of a scoring system for UTUC grading. Besides different combinations of CLE criteria, different allocations of points for high-grade CLE features were evaluated for the scoring systems. High-grade features could score either two

or three points while low-grade features scored 1 point and undefined features were allocated 0 points. The diagnostic ability of the different scoring systems was evaluated by receiver operating characteristic (ROC) curve analysis with area under the curve (AUC) testing against the null-hypothesis (AUC=0.5). Additionally, DeLong testing was performed for pairwise comparison of the AUC of the different scoring systems. Validation of the proposed scoring systems could not be performed due to the limited amount of data.

Statistical analyses were performed using SPSS Statistics version 24 and MedCalc V18.6.

Results

Patient and tumour characteristics

From August 2016 until March 2018, a total of 150 procedures in 73 individual patients were included. CLE imaging was performed in 68 procedures, in which upper urinary tract lesions were visualized. The CLE recordings of 53 papillary UTUCs from 51 procedures in 36 patients were included in the final analysis (Figure 1). Patient and tumour characteristics are presented in Table 1.

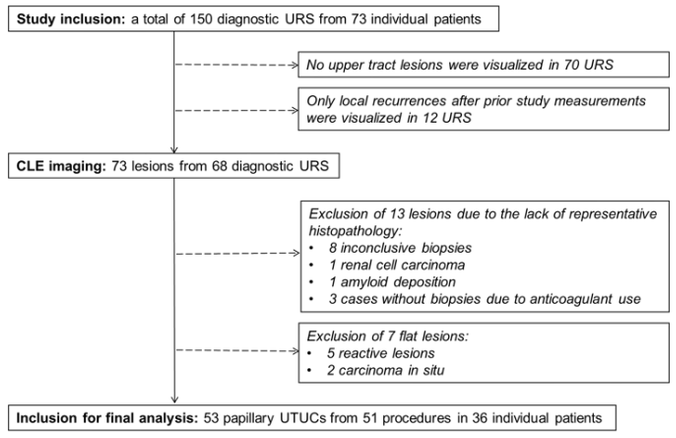

Figure 1: Flow diagram of inclusion for final analysis.
Figure legends: URS = ureterorenoscopy, UTUC = upper tract urothelial carcinoma

Table 1: Patient and tumour characteristics.

A. Patient characteristics		**N=36**
Age in years, mean (sd)		70 (11)
Gender, n (%)	Female	11 (31)
	Male	25 (69)
Prior UTUC, n (%)		18 (50)
B. Tumour characteristics		**N=53**
Laterality of disease, n (%)	Left	27 (51)
	Right	26 (49)
Tumour location, n (%)	Ureter	23 (43)
	Pyelocalyceal system	30 (57)
Tumour diameter in mm, median (absolute range)		8 (2 – 100)
Tumour grade WHO 2004, n (%)	Low-grade	34 (64)
	High-grade	19 (36)

Table legends: sd = standard deviation, UTUC = upper tract urothelial carcinoma

Prevalence of CLE criteria

All the previously described CLE criteria were identifiable in varying proportions of low-grade and high-grade UTUCs. The prevalence of the CLE criteria per tumour grade of all 53 papillary UTUCs are presented in Figure 2. The most prevalent CLE features between low-grade and high-grade UTUCs were: organization versus disorganization of the cellular architecture; monomorphism versus pleomorphism of cells; and cohesiveness versus discohesion of cells. Representative examples of the identified CLE features are presented in Figure 3.

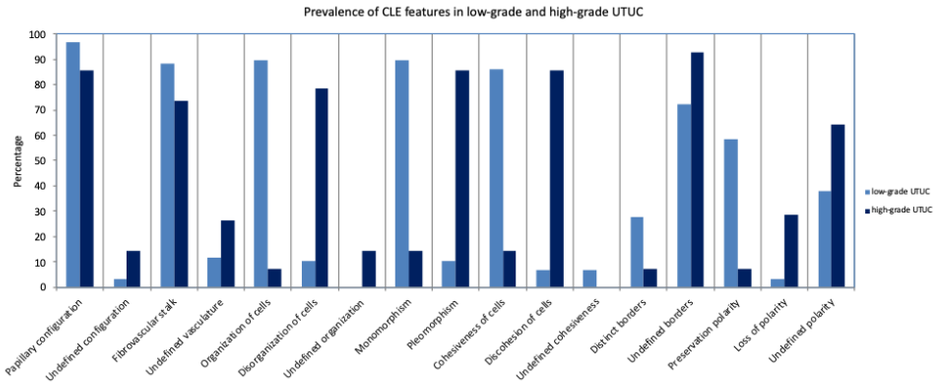

Figure 2: Prevalence of CLE features in low-grade and high-grade UTUC.

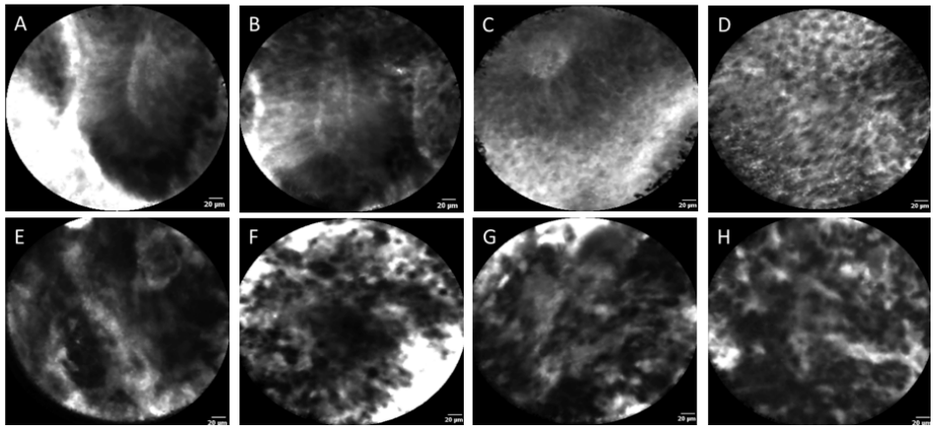

Figure 3: In-vivo CLE images of low-grade (A – D) and high-grade (E – H) upper urinary tract urothelial carcinoma: A & B) cohesive, papillary configuration with fibrovascular stalk; C) fibrovascular stalk with preservation of polarity; D) cohesive and organized cellular architecture of monomorphic cells with distinct cell borders; E) fibrovascular stalk and disorganized cellular architecture; F) discohesive pleomorphic cells; G & H) disorganization of pleomorphic cells.

Diagnostic accuracy estimates

For the complete cohort, the assessment of all the 7 CLE criteria resulted in a correct grade prediction in 38 of the 53 papillary UTUCs (72%, 95% CI 58–83%). The sensitivity for low-grade UTUC of the complete cohort was 77% (95% CI 59–89%) with a specificity of 63% (95% CI 38–84%). In five low-grade (15%) and five high-grade (26%) tumours, the CLE recordings were rated as non-diagnostic due to insufficient CLE image quality for CLE feature identification. When excluding the non-diagnostic recordings, assessment of all the CLE criteria resulted in a correct grade prediction in 38 of the 43 UTUCs (88%, 95% CI 57–92%) with a sensitivity for low-grade UTUC of 90% (95% CI 73–98%) and a specificity of 86% (95% CI 78–98%). The sensitivity, specificity, PPV, and NPV of the individual CLE criteria are presented in Table 2. The CLE criteria of cellular organization, cellular morphology and cellular cohesiveness achieved the highest diagnostic accuracy estimates.

Inter-rater agreement

The inter-rater percentage agreement for CLE criteria assessment and CLE-based grading between the two raters are presented in Table 2. The inter-rater percentage agreement was acceptable for all the CLE criteria except for cellular cohesiveness.[20]

Table 2: Inter-rater percentage agreement for each CLE feature and overall CLE-based grading, and estimations for the sensitivity/specificity and the positive predictive value/negative predictive value with regard to low-grade UTUC.

CLE criteria	*CLE feature variables*	Calculations with the total cohort including (n=53)					Calculations with the diagnostic cases only (n=43)				
		Inter-rater percentage agreement	Sensitivity in % [CI]	Specificity in % [CI]	PPV in % [CI]	NPV in % [CI]	Inter-rater percentage agreement	Sensitivity in % [CI]	Specificity in % [CI]	PPV in % [CI]	NPV in % [CI]
Papillary configuration	*Papillary/not papillary/undefined*	91	91 [76-98]	21 [6-46]	67 [62 – 73]	57 [52-78]	95	97 [82-100]	14 [2-43]	70 [65-75]	67 [17-95]
Vasculature	*Fibrovascular stalk/large vessels/undefined*	87	85 [69-95]	16 [3-40]	64 [59-70]	38 [14-69]	86	100 [88-100]	21 [5-51]	73 [67-78]	100 [33-100]
Cellular organization	*Organized/disorganized/undefined*	93	77 [59-89]	58 [34-80]	76 [65-85]	58 [40-74]	93	90 [73-98]	79 [49-95]	90 [76-96]	79 [59-92]
Cellular morphology	*Monomorphic/pleomorphic/undefined*	91	77 [59-89]	63 [38-84]	79 [67-87]	60 [43-75]	91	90 [73-98]	86 [57-98]	93 [78-98]	80 [57-92]
Cellular cohesiveness	*Cohesive/discohesive/undefined*	70	76 [59-89]	74 [49-91]	84 [71-92]	64 [47-77]	74	86 [68-96]	86 [57-98]	93 [77-98]	75 [54-88]
Cellular polarity	*Polarity/loss of polarity/undefined*	83	50 [32-68]	21 [6-46]	64 [50-77]	19 [8-37]	79	59 [39-76]	29 [8-58]	63 [52-73]	25 [12-46]
Definition of borders	*Distinct/undefined*	83	24 [11-41]	95 [74-100]	89 [52-98]	41 [36-46]	79	28 [13-47]	93 [66-100]	89 [53-98]	38 [32-45]
CLE-based grading	***Low-grade/high-grade/non-diagnostic***	**93**	**77 [59-89]**	**63 [38-84]**	**79 [67-87]**	**60 [43-75]**	**93**	**90 [73-98]**	**86 [57-98]**	**93 [78-98]**	**80 [57-92]**

Table legends: CI = confidence interval, CLE = confocal laser endomicroscopy, NPV = negative predictive value, PPV = positive predictive value.

Proposal of a CLE-based scoring system for UTUC grading

Two scoring systems with an allocation of either 2 or 3 points for high-grade features were proposed for the CLE criteria of cellular organization and cellular morphology (labeled as '2 criteria — 2 points' and '2 criteria — 3 points'). Two additional scoring systems were proposed by adding cellular cohesiveness to the above-mentioned systems (labeled as '3 criteria — 2 points' and '3 criteria — 3 points'). The ROCs of each scoring system are presented in Figure 4. DeLong testing resulted in a statistically significant difference for the pairwise comparison of the AUCs of the '3 features — 2 points' and the '2 features — 2 points' scoring system only (p = 0.045). The individual AUC of each scoring system with the optimal cutoff and corresponding sensitivity and specificity are presented in Table 3. The '3 features — 3 points' CLE-based scoring system, as illustrated in Figure 5, yielded the highest diagnostic ability.

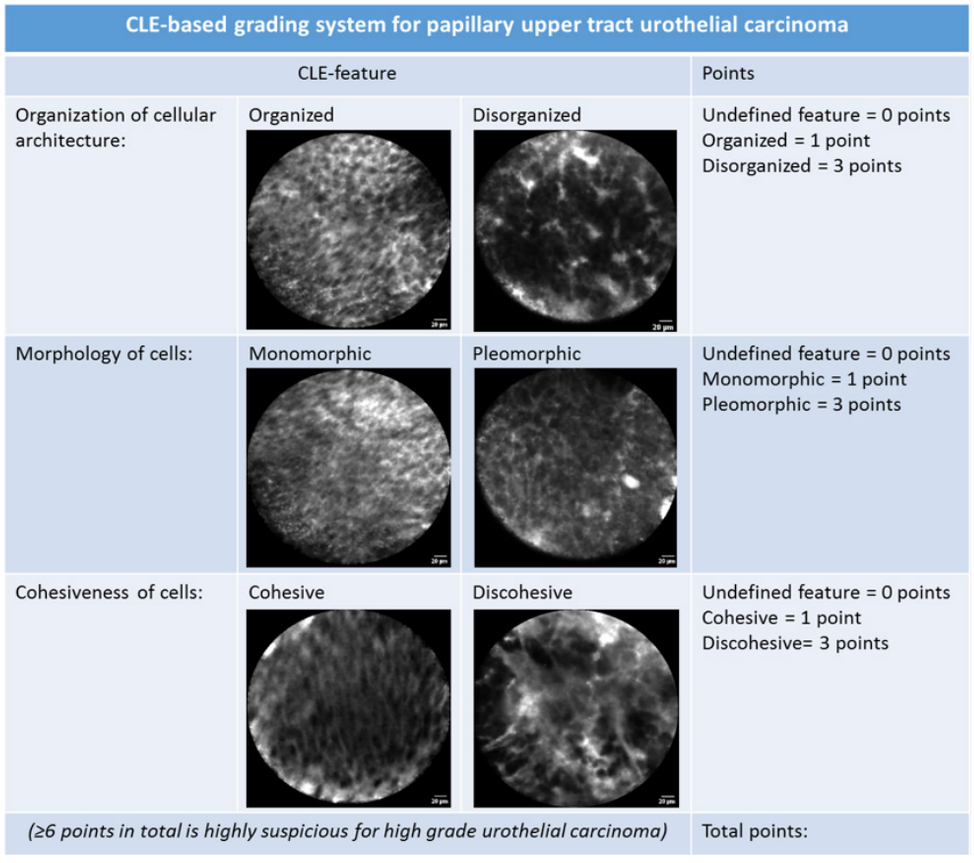

Figure 4: CLE-based scoring system for UTUC grading.

Table 3: Overview of the diagnostic abilities of the assessed CLE-based scoring systems.

CLE based scoring system	Area under the curve [CI]	Significance level of the AUC	Cut-off value for low-grade UTUC	Sensitivity at the cut-off	Specificity at the cut-off
'2 criteria – 2 points'	0.81 [0.66 – 0.91]	<0.001	≤2	90 [73-98]	79 [49-95]
'2 criteria – 3 points'	0.84 [0.70 - 0.94]	<0.001	≤2	90 [73-98]	86 [57-98]
'3 criteria – 2 points'	0.90 [0.77 - 0.97]	0.045	≤3	86 [68-96]	86 [57-98]
'3 criteria – 3 points'	0.93 [0.81 - 0.99]	<0.001	≤3	86 [68-96]	93 [66-100]

Table legends: AUC = area under the curve, CI = confidence interval, CLE = confocal laser endomicroscopy, UTUC = upper tract urothelial carcinoma

Discussion

With this study we confirm that the previously reported CLE criteria for UCB are also applicable for ureterorenoscopic CLE images of papillary UTUC. However, the visual appearance and the prevalence of the CLE criteria differ from UCB. Although preliminary, the assessment of CLE criteria allows for accurate identification of the histopathologic grade in papillary UTUC. The most prevalent CLE criteria with also the highest diagnostic potential for UTUC grading are cellular organization, morphology and cohesiveness of cells.

The difference in visual appearance and prevalence of the CLE criteria in UTUC compared to CLE in UCB can be explained by the different optical system of the ureterorenoscopic CLE probe. The decreased ability to discriminate between two objects (inferior resolution) and the greater superimposition of cellular structures (larger depth of the confocal plane) results in inferior definition and sharpness of the ureterorenoscopic CLE images.[17] Consequently, cell borders were not clearly defined and therefore not assessable in the majority of low-grade and high-grade UTUC. As a result, the diagnostic potential of this criterion for tumour grading, as described by Liem et al for UCB, could not be confirmed for UTUC.[14] Similarly, the state of cellular polarity was often undefined in CLE images of UTUC. The papillary configuration and fibrovascular stalks were identifiable in almost all ureterorenoscopic CLE recordings. Since these criteria are by definition present in papillary UTUC, they do not aid UTUC grading.

Chang et al suggested that tortuous vessels are characteristic for high-grade UC.[10] In our study, the definition of tortuous vessels was deemed subjective and could not be identified accurately in ureterorenoscopic CLE images.

The inter-rater agreement for the individual CLE criteria was acceptable except for cellular cohesiveness. The inter-rater percentage agreement of this criterion

was slightly below the threshold for acceptable agreement. The results for the inter-rater agreement are in line with the literature.[12,14] Also the estimates of the sensitivity and specificity for CLE-based grading are in line with the results by Breda et al.[12] As such, he proposed CLE criteria enable reproducible and accurate assessment of the UTUC grade.

The proposed scoring system based on cellular organization, morphology and cohesiveness with an allocation of 3 points for high-grade features resulted in the highest sensitivity and specificity for UTUC grading. However, the reproducibility of cellular cohesiveness was below the threshold for acceptable agreement, which could limit the diagnostic ability of the scoring system. The scoring system based on only cellular organization and morphology with an allocation of 3 points for high-grade features resulted in a very similar ROC and AUC. Yet, despite a slightly higher sensitivity, the scoring system based on two criteria resulted in a lower specificity than the system based on three criteria. In comparison to the diagnostic accuracy of UTUC grading based on all CLE criteria of the present study and of Breda et al, both scoring systems yield similar values.[12] Nonetheless, both scoring systems required external validation. But reducing the number of CLE criteria and quantifying the significance of CLE features towards tumour grading can contribute towards simplification and standardization of CLE image assessment. This would enhance the clinical applicability of CLE for intra-operative grading of UTUC.

In the present study, the diagnostic yield of CLE-based grading was comparable to the diagnostic yield of ureterorenoscopic biopsies.[4–6,8] In comparison with the study by Breda et al for intraoperative CLE-based classification of UTUC, our diagnostic yield for CLE was lower.[12] In contrast to our study, however, the raters of Breda et al were not blinded to the ureterorenoscopic appearance of the imaged tumours. Potentially, the learning curve of CLE application and interpretation might have influenced the diagnostic yield. But the users and raters of the current study were already familiar with the technique. Familiarization with application and interpretation of CLE was achieved within a small number of cases beforehand. Another point of concern might be the procedural application of fluorescein. Investigating the pharmacokinetics of fluorescein with regard to urothelium may help to optimize the procedural protocol. Furthermore, technical improvements the CLE probe's optics, e.g. reduction of the depth of confocal plane, can contribute to superior image quality. Moreover, the durability of the CLE probe might also be a point of concern as the image quality seemed to deteriorate with cumulative use.

The next step in the development of CLE as a tool for real-time tumour grading requires a powered analysis of its diagnostic accuracy during ureterorenoscopy,

preferably in combination with a validation of the proposed scoring system. Owing to the low incidence of UTUC, a joint multicentre approach is required to achieve powered studies within a reasonable timeframe to fully grasp the potential of CLE for UTUC. More data on this topic is also needed for the development of convolutional neural networks for computer aided CLE assessment. Moreover, in theory, confocal laser endomicroscopy allows for in-vivo assessment of the complete tumour and therewith may avoid undergrading with regard to intra-tumoral heterogeneity or sampling error.[5,21,22] Moreover, CLE-based grading of papillary UTUC in-vivo may allow for accurate intra-operative risk stratification and hence facilitation of immediate treatment selection. This could lead to a reduction in the number of subsequent URS, surgery time and health-care costs.

Limitations

First, the histopathologic findings of co-localized ureterorenoscopic biopsies were used as the reference standard for comparison. The histopathologic grade of ureterorenoscopic biopsies may not be accurate in comparison to the histopathology of surgical resections due to possible grade heterogeneity, sampling error or subjectivity of the histopathologic assessment.[4,5,21,22] On the other hand, biopsies allow for superior macroscopic co-localization of the histopathologic assessed tissue and the imaged region with the index test than resection specimens. Yet more importantly, relying on the histopathologic grade of ureterorenoscopic biopsies did not allow for a direct comparison of the diagnostic yield and accuracy between CLE imaging and biopsies. Studies of comparative accuracy are required to identify the potential role of CLE for the current diagnostic pathway.[23]

The proposed scoring system was based on univariate analysis. The accuracy of the scoring system could be improved with multivariate statistics and an increased sample size.[24] Moreover, the proposed scoring system requires validation.

Next, the histopathologic assessment of biopsies was performed by a single uropathologist. While this single-rater approach avoided inter-rater variability, the most accurate histopathologic grading would result from an expert-panel consensus.[25]

A technical limitation of CLE is the requirement of a fluorescent contrast agent. Besides adding an extra preparation step, the ureterorenoscopic vision after fluorescence application may be hampered. The vision can be improved by flushing saline through the ureteroscope, but this is time consuming and should be minimalized to avoid high intra-renal pressures.

This study only investigated papillary UTUCs. The potential of CLE for the identification of CIS amongst flat lesions remains to be investigated.

Conclusions

CLE allows for accurate grading of papillary UTUC with the previously described CLE criteria for urothelial carcinoma. The most prevalent and discriminating CLE criteria in papillary UTUC are cellular organization, morphology and cohesiveness. The proposed scoring system based on these criteria for UTUC grading may allow for a more quantifiable and simplified approach at a similar diagnostic accuracy. External validation of the proposed scoring system is required.

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Chapter 8

Fluorescence In Situ Hybridization in 1 mL Samples of selective Upper Tract Urine for the Detection of Upper Tract Urothelial Carcinoma: A Feasibility Study

J.E. Freund, E.I.M.L. Liem, C.D. Savci-Heijink, T.M. de Reijke
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List of Abbreviations

CEP – chromosome enumeration probe
FISH – fluorescence in situ hybridization
KSS – kidney sparing surgery
LSI – locus specific identifier
NPV – negative predictive value
PPV – positive predictive value
RNU – radical nephroureterectomy
TPS –The Paris System
UCB – urothelial carcinoma of the bladder
URS – ureterorenoscopy
UT – upper urinary tract
UTUC – upper tract urothelial carcinoma

Abstract

Kidney-sparing surgery of upper tract urothelial carcinoma (UTUC) requires a stringent follow-up with frequent ureteroscopies. Triage testing could reduce the number of follow-up ureteroscopies and hence minimize the invasiveness of follow-up. The use of urine-based markers for triage seems appealing but should be feasible with selective urine from outpatient cystoscopy to maximize the reduction of invasiveness. In this study, the feasibility of UroVysion® fluorescence in situ hybridization (FISH) for the detection of UTUC in 1 mL of selective urine is investigated.

Ten consecutive patients with biopsy-proven UTUC and five patients with negative diagnostic ureteroscopy findings were included in this case-control study. During ureteroscopy, 1 mL of selective urine was collected passively with a ureteral splint for Urovysion® FISH. The FISH rater was blinded to any clinical information.

The results of FISH were compared to the findings of concomitantly collected selective urine cytology and the patients' UTUC status. FISH was feasible in all samples with a sensitivity of 90% and a specificity of 80% for UTUC. In comparison, selective cytology resulted in a diagnostic yield of 87% with a sensitivity of 80% and a specificity of 67%.

In conclusion, UTUC detection is feasible with FISH in 1 mL of passively collected selective urine. Thus, from a technical point of view, FISH could be used as an outpatient triage test to decide if follow-up ureteroscopy is necessary after kidney-sparing surgery of UTUC. Evaluation of the diagnostic accuracy of FISH for the suggested pathway deserves further attention.

Introduction

Upper urinary tract urothelial carcinoma (UTUC) arises from the urothelial lining of the ureter and pyelocaliceal system. The peak incidence of the disease is at 70 to 90 years of age. Ureterorenoscopy (URS) under general anaesthesia is the corner stone for UTUC diagnosis as it enables the visualization of the upper urinary tract (UT) urothelium and the acquisition of tissue biopsies. Histopathologic assessment of suspicious lesions is essential for primary diagnostics and risk-stratification for adequate treatment selection.[1]

High-risk UTUC is generally treated by radical nephroureterectomy (RNU). In low-risk disease (per definition low-grade UTUC), kidney-sparing surgery (KSS) is the primary choice of treatment.[1] KSS may be offered in the form of ureteroscopic laser fulguration or segmental ureterectomy for ureteral tumours. These treatment options yield a similar cancer specific survival as RNU in low-risk cases.[1] However, high local recurrence rates after KSS are a point of concern that asks for a stringent follow-up.[1] Optimal follow-up includes frequent URS under general anaesthesia, which is demanding for the elderly UTUC population. Triaging before follow-up URS could lead to a reduction in the number of invasive diagnostic interventions and hence improvements in the quality of life and reduction of health care costs after KSS.

Urine-based diagnostics are an appealing option for triage testing in the follow-up after KSS. However, standard cytological assessment of selectively collected urine from the upper urinary tract is not suitable as a triage test for the suggested pathway. This is because selective urine cytology lacks sensitivity for low-grade UTUC and KSS is especially offered to low-grade disease.[2–4] Yet, specific urine-based markers could be more suitable for triage testing in the follow-up after KSS.

UroVysion® FISH is an approved urine-based cytogenetic test for the detection of urothelial carcinoma of the bladder (UCB).[5] Several studies have already investigated the role of FISH in diagnosing UTUC and reported promising results.[6–8] As such, UroVysion® FISH may qualify as a triage test for the follow-up after KSS. However, as the manufacturer advices a minimum urine volume of 32 mL for FISH analysis, the feasibility of FISH in small volumes of selective urine that can be collected passively during outpatient cystoscopy from the upper urinary tract has not been explored yet.

In the present study, the feasibility of UroVysion® FISH for the detection of UTUC in 1 mL of passively collected selective upper tract urine is investigated. To our knowledge, this is the first study that assessed the use of UroVysion®

FISH in passively collected upper tract urine in a manner that could be applied during outpatient cystoscopy without general anesthesia. Additionally, the results of FISH are compared with the findings of standard cytology from concomitantly collected upper urinary tract urine.

Methods

Patients and sampling

From September 2017 until March 2018, ten consecutive patients with histopathological confirmed UTUC and five consecutive patients with negative findings during URS were included prospectively in this case–control study.

The UTUC group consisted of adult patients who underwent flexible digital URS (Olympus V2 or Karl Storz Flex XC) with ureteroscopic biopsies of UTUC and selective urine cytology sampling for standard clinical care. The presence of UTUC was confirmed by ureteroscopic biopsies. Biopsies were taken with Piranha® forceps or French Zero-tip baskets (both Boston Scientific, Massachusetts, USA). Histopathologic assessment was performed according to the local clinical protocol by an uropathologist (C.D.S.), who was blinded for FISH results.

The control-group consisted of adult patients in whom the presence of UTUC was ruled out by visual assessment of the complete upper urinary tract by flexible digital URS (Olympus V2 or Karl Storz Flex XC). Selective urine cytology sampling was performed for standard clinical care.

Patients were excluded in case of bladder cancer within 3 months prior to or at the time of URS with selective urine sampling. Additionally, patients were excluded for the control-group if UTUC was present in either UT within 3 years prior to the selective urine sampling.

The institutional review board granted a waiver for this case–control feasibility study as no additional activities in human subjects were involved.

Procedure

Urine sampling was performed under general anesthesia in the operation theater at the start of the diagnostic URS. According to the standard clinical protocol, patients received 20 mg furosemide, intravenously approximately 20 min prior to urine sampling to stimulate urine production. Selective urine sampling was performed passively via a six French ureteral splint, which was placed under fluoroscopy through a rigid cystoscope (Olympus) or semirigid ureteroscope (Karl Storz) in the distal/mid-ureter. 1 mL of the sampled UT urine was used for FISH analysis and the remaining volume of at least 3 mL was used for standard cytological assessment. Contrast-based retrograde fluoroscopy and introduction of the ureteroscope were performed after the described urine sampling had been completed.

The FISH sample was immediately mixed with 0.5 mL Carbowax (polyethylene glycol) for fixation. Cytospins were made within 24 hours, which resulted in two slide preparations per sample. The slide preparations were fixed with Carnoy's solution (3:1 methanol:glacial acetic acid) and stored at − 20 °C until FISH was performed.

Selective urine cytology was processed and assessed according to the standard clinical protocol by a cyto-technologist and uropathologist, both blinded for FISH results.

FISH protocol and interpretation

All slide preparations were analyzed using the UroVysion® FISH bladder cancer assay (Abbott Molecular, Illinois, USA). This FISH assay enables the visualization of molecular alterations (aneuploidy of chromosome 3, 7, and 17; loss of locus 9p21) commonly seen in UCB. The pre-mixed and pre-denatured UroVysion® probe mixture consists of four fluorescent labeled nucleic acid probes [Chromosome Enumeration Probe (CEP) 3, CEP 7, CEP 17, and locus specific identifier (LSI) 9p21]. With excitation of the hybridized probes, the number of the specific chromosome copies is visualized for enumeration.

The slides were pre-treated with the UroVysion® Vysis pre-treatment kit. FISH was performed according to the manufacturer's protocol with overnight hybridization of the probe mixture with the ThermoBrite System (Abbott) (2 min at 73 °C, 12–16 h at 37 °C). For post-hybridization, the slide preparations were washed in 2X SSC/0.1% NP-40 at room temperature until the coverslips were floated off, 0.4X SSC/0.3% NP-40 at 72 °C for 2 min, and lastly again in 2X SSC/0.1% NP-40 at room temperature for 1 min. The nuclei were counterstained with DAPI (40,6-diamidino-2-phenylindole).

A single, trained observer (J.E.F.), who was blinded for clinical data, performed enumeration of FISH signals. For enumeration, a fluorescence microscope (Leica DM 5500B) was used with the prescribed filters: A4 blue for DAPI, TX2 red for CEP 3, L5 green for CEP 7, SAQ aqua for CEP 17, and SGO gold for LSI 9p21. Specimens were considered to be positive for FISH if among 25 morphologically abnormal cells (large nuclear size, irregular nuclear shape, patchy DAPI staining) ≥ 4 cells had a gain of 2 or more chromosomes (3, 7, or 17), or ≥ 12 cells had a loss of both copies of LSI 9p21.[9]

Results

The patient characteristics of the UTUC group and the control-group are presented in Table 1. Table 2 lists the distribution of FISH and cytology findings of the UTUC group and the control-group. Sufficient cells were present in all 15 FISH preparations for FISH enumeration, resulting in a diagnostic yield of 100%. The diagnostic yield for selective urine cytology was 87% as two of the 15 UT cytology samples were interpreted as inconclusive.

Table 1: Patient characteristics

	UTUC group (n=10)	Control group (n=5)
Gender: women/men	3/7	2/3
Age in years, *median (range)*	70 (46 – 90)	61 (39 – 71)
Prior UTUC history, *n*	6	3
Highest grade of former UTUC (WHO 2004), *n*		
Low-grade	1	2
High-grade	5	1
Prior UCB, *n*	5	1
Highest grade of former UCB (WHO 2004), *n*		
Low-grade	3	1
High-grade	2	0
Time since last UCB in months, *median (range)*	72 (4-86)	166 (na)

Table legends: na = not applicable, UCB = urothelial carcinoma of the bladder, UTUC = upper urinary tract urothelial carcinoma.

As shown in Table 2, nine of the ten FISH assays were positive (sensitivity 90%) in the UTUC group, while eight of ten selective urine cytology findings were positive for low-, or high-grade UTUC (sensitivity 80%). In the control-group, four FISH assays were negative, yielding a specificity of 80%. For selective urine cytology, two of three conclusive findings were negative for UTUC, resulting in a specificity of 67%.

Table 2: Findings per case.

Case	Tumour location	Histologic grade from biopsy (WHO 2004)	Selective urine cytology finding	FISH interpretation
1	Distal ureter	HG	No malignancy	+
2	Upper pole and interpolar	HG	HG	+
3	Upper pole	LG	LG	+
4	Renal pelvis	LG	LG	+
5	Renal pelvis	LG	HG	+
6	Distal ureter and renal pelvis	HG	HG	+
7	Distal ureter	HG	HG	+
8	Upper pole	HG	HG	+
9	Renal pelvis	LG	No malignancy	–
10	Distal ureter	HG	LG	+
11	No UTUC visualized	Not applicable	Inconclusive	–
12	No UTUC visualized	Not applicable	Inconclusive	–
13	No UTUC visualized	Not applicable	LG	–
14	No UTUC visualized	Not applicable	No malignancy	+
15	No UTUC visualized	Not applicable	No malignancy	–

Table legends: HG = high-grade, LG = low-grade, + = positive for UTUC, – = negative for UTUC.

Discussion

UTUC detection is feasible with UroVysion® FISH in one mL of selective urine. This enables FISH to detect UTUC in small volumes of UT urine that could be collected passively via a ureteral splint during outpatient cystoscopy. From a technical point of view, FISH may, therefore, qualify as a triage test in the outpatient setting to reduce the number of URS in the follow-up after KSS. Limiting the number of invasive URS in the follow-up after KSS is desirable for quality of life improvements in the primarily elderly population of UTUC. Moreover, reducing surgical follow-up under general anaesthesia with hospitalization might also reduce procedure-associated complications and health care costs.

Yet, the implementation of FISH as a triage test is dependent on its diagnostic accuracy. In this study, the diagnostic accuracy seems promising but is preliminary. Comparison with the current literature seems somewhat arbitrary

when considering the different cut-off values and various types of urine sampling methods investigated (voided, UT brushing, UT washing, and passively collected UT urine). Nevertheless, the sensitivity and specificity of FISH for UTUC detection in this feasibility study are in line with the reported range in the literature (35–88% and 78–96%, respectively).[6,10–13] We believe that further studies to investigate the diagnostic accuracy of FISH as a triage test for ureteroscopy in the follow-up after KSS of UTUC are warranted.

In the present study, the diagnostic accuracy of FISH seems superior to the diagnostic accuracy of selective UT urine cytology for UTUC detection. Also, in the literature, urine cytology for UCB and UTUC is known to have a lower sensitivity at a comparable or higher specificity than FISH.[4,13–16] But, especially for low-grade urothelial carcinoma, the diagnostic yield of urine cytology is limited.[4] This hampers the suitability of standard cytological assessment as a triage test for the follow-up after KSS, which is generally performed in low-grade UTUC only. Furthermore, challenges in distinction of reactive and neoplastic causes of atypia lead to inaccurate reporting of urine cytology.[17] Inter-observer variability may also influence the diagnostic accuracy of both urine-based tests. Despite the fact that the interpretation of FISH is dependent to subjectivity, FISH results may be more quantifiable than cytology.[18]

A limitation of the current study is the small sample size and the case-control study design. This might lead to an inaccurate estimation of the diagnostic accuracies. Due to the highly selected patient cohort, the influence of concomitant UCB on FISH results and cytology findings remains to be investigated. In case of concomitant UCB, ureteral reflux of UCB cells may lead to higher false positive rates.[8] Moreover, the role of ureter splint location for selective urine collection with regard to ureteral reflux and tumour location has not been identified yet.

Next, FISH interpretation was only performed according to the manufacturer's instructions. Interpretation with cut-off values for enumeration other than specified by the manufacturer might yield different diagnostic accuracies for UTUC detection.[19] Additionally, the lack of multiple raters does not facilitate the assessment of inter-observer variability of FISH interpretations or diagnostic accuracy range calculations.

Likewise, new and potentially superior interpretation methods for reporting urine cytology, such as The Paris System (TPS), were not included in this study. Consequently, the diagnostic accuracy of selective urine cytology might be underestimated when considering the promising results with TPS.[20]

To identify the possible role of FISH for the follow-up of KSS, the diagnostic accuracy of FISH in selective urine from outpatient cystoscopy should be investigated in powered studies. Such studies may also facilitate the evaluation of different cut-off values for FISH interpretation to improve the diagnostic accuracy for UTUC detection. In addition, a comparative assessment of multiple urine-based markers may be performed directly to investigate further optimization of the diagnostic pathway.

Conclusions

UTUC detection is feasible with UroVysion® FISH in one mL of passively collected upper tract urine. From a technical point of view, FISH could be used as an outpatient triage test to decide if follow-up ureteroscopy following kidney-sparing surgery of UTUC is necessary. The initial estimate of the diagnostic accuracy of FISH seems comparable to the current literature of FISH assessment in selective urine samples of greater volumes. Further evaluation of the diagnostic accuracy of FISH for the suggested diagnostic pathway should be stimulated.

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Chapter 9

General discussion and future perspectives

List of abbreviations

CLE – confocal laser endomicroscopy
FISH – fluorescence in situ hybridization
OCT – optical coherence tomography
PALGA – Dutch nationwide network and registry of histo- and cytopathology
UTUC – upper tract urothelial carcinoma

General discussion and future perspectives

Upper tract urothelial carcinoma (UTUC) is a disease of low, but increasing prevalence.[1] In case of diagnostic uncertainty using radiology and urine cytology, ureterorenoscopy with ureterorenoscopic biopsies are essential to the diagnostic approach. Additionally, the histopathologic grade from ureterorenoscopic biopsies is a key factor in the risk-stratification of UTUC for optimal treatment selection. To improve the current diagnostic paradigm, this thesis aimed to provide more insight into the shortcomings of the current diagnostic approach to UTUC. Second, this thesis investigated the diagnostic potential of optical techniques to overcome these shortcomings.

Chapters 2 and 3 cover the first aim of this thesis, confirming the diagnostic limitations of ureterorenoscopic biopsies and portraying the lack of accuracy of grade predictions based on the visual appearance during digital ureterorenoscopy. These findings justify the efforts to improve the current diagnostic approach to UTUC. **Chapters 4 to 8** address the second aim of the thesis, in which the diagnostic potential of optical coherence tomography (OCT), confocal laser endomicroscopy (CLE) and fluorescence in situ hybridization (FISH) for the diagnosis and grading of UTUC are highlighted.

With regard to the IDEAL framework, the current state of development of CLE for grading of UTUC has reached the transition from the exploration stage (stage 2b) to the assessment stage (stage 3).[2] Larger, and hence multicentre studies are needed to confirm the promising diagnostic results. Larger studies will also allow for the assessment of the diagnostic potential of CLE for flat lesions, as the present cohort studies were too limited to include a reasonable number of flat lesions. Future studies should also include the assessment of CLE images in real-time during ureterorenoscopy. Notably, so far, little attention has been paid to whom and how the assessment of the CLE images at real-time during ureterorenoscopy should be executed. Besides investigating the learning curve of CLE image assessment, we also need to address the diagnostic accuracy with intra-, and inter-observer variability for different observers in different settings, e.g. performing endourologists versus pathologists or trained analysts without surgical tasks.

To date, the use of OCT in the upper urinary tract has reached the developmental stage (2a) of the IDEAL framework.[2] The number of studies is still limited despite promising results.[3] The financial aspect of the OCT probe might be a limitation for further studies, even though OCT has been implemented successfully in cardiology for intravascular assessment of blood vessels. Furthermore, the development of a forward-looking OCT probe is required to enable imaging in the renal calices. Technical improvements may also stimulate future studies. Besides UTUC grading, future studies should also continue investigating OCT for UTUC staging. In view of this, further development of OCT for the diagnostic process of UTUC should be appealing as it can provide two histopathologic factors of prognostic value with a single measurement in real-time.

Next, the integration of OCT and CLE into a single optical imaging device for multimodal optical biopsies should be explored by biomedical engineers. Combining the diagnostic potential of different lasers in a single device could boost the diagnostic accuracy of these techniques, potentially without an increase in patient burden. Moreover, computer-aided assessments of OCT and CLE should provide further improvements in the diagnostic accuracy for the diagnosis and grading of UTUC.[4]

The use of FISH in small amounts of upper urinary tract urine for the detection of UTUC is at IDEAL stage 1, as the first feasibility study is presented in this thesis.[2] Besides being feasible, FISH yielded a better estimated diagnostic accuracy for the detection of UTUC compared to standard cytology. Consequently, from a technical point of view, FISH qualifies as a triage test during outpatient cystoscopy in the follow-up after kidney-sparing treatment. This finding should stimulate prospective cohort studies to investigate if the diagnostic accuracy of FISH allows for active surveillance, and thereby reduce the number of follow-up ureterorenoscopies and improve the quality of life of UTUC patients.

The low prevalence of UTUC, however, might be a hindrance to any further development of the investigated techniques, as the completion of single-centre studies with statistical power within a contemporary timeframe is problematic. Therefore, we must promote national and international collaborations to initiate multicentre studies to press ahead with the development of optical biopsies for an improved diagnostic approach to UTUC.

In the light of the low prevalence of the disease, retrospective cohort studies are highly useful to study disease specific factors. Owing to the pathology-initiated 'nationwide network and registry of histo- and cytopathology in the Netherlands' (PALGA), this thesis presents the first nationwide and currently largest cohort study to confirm the diagnostic limitations of ureterorenoscopic biopsies. The PALGA should be an example for other nationwide registries as it collects every histo- and cytopathology report with its clinical data from all hospitals in the Netherlands since 1991. Thereby, the PALGA is a unique source for clinical auditing and external quality assessment. Similarly, the PALGA

facilitates high-volume cohort studies to address pathologic and clinical aspects of potentially all diseases. Yet, as described in **Chapter 2**, retrospective studies have several limitations. A major limitation of the PALGA is the heterogeneity of data and varying availability of specific data in the clinical data and histopathologic reports. Despite the possibility of coupling the PALGA with the 'Netherlands Cancer Registry', this specific limitation still introduces missing data, which hampers the exploitation of the full potential of a nationwide database. Therefore, besides encouragements for the implementation of nationwide registries in other countries, we should seek to implement standardization of clinical data and histopathologic reports to improve the quality of registered data. The standardization of these reports should be defined jointly in international and multidisciplinary expert panels to warrant collaborations and comparison of data. Protocolization of the reported data is also desirable with regard to computer-automated identification and analysis of data to facilitate an efficient methodology of high-volume retrospective cohort studies in the near future. Yet, it is essential that standardized protocols leave room for case specific annotations and that the completeness of content does not come at the cost of work efficiency. Furthermore, in the light of the progressive implementation of digital pathology and the development of computer-automated image analyses, we should strive after the integration of digital histopathology in the abovementioned registries to exploit the full potential for future studies.

Lastly, with regard to the low prevalence of the disease, centralization of UTUC care may improve the homogeneity of data. More importantly though, the centralization of care may improve patient outcomes. Centralizing the diagnostic work-up in the hands of dedicated endourologists and under the watchful eye of uropathologists may result in improved diagnostic yield and higher diagnostic accuracies with lower interobserver variability. Ultimately, the best treatment starts with the best diagnosis. Moreover, centralization of the therapeutic endourologic and surgical care will benefit oncologic outcomes in this low prevalent disease, too.

In this thesis, a major point for discussion may be the definition of the reference standard for the diagnostic accuracy studies. Based on the findings in **Chapter 2**, one may argue that ureterorenoscopic biopsies may not be the optimal reference standard for diagnostic accuracy studies when assessing the histopathologic grade. The rate of upgrading of ureterorenoscopic biopsies in comparison with the final resection may introduce a diagnostic error. This limitation applies to **Chapters 3, 5, 7 and 8** of this thesis. In these chapters, the index test was compared to the diagnosis and the histopathologic grade of co-localized ureterorenoscopic biopsies. Yet, the setting of these studies

in a tertiary hospital with endourologic expertise and a dedicated uropathologist probably has benefited the concordance of ureterorenoscopic biopsies. Furthermore, the discordance of ureterorenoscopic biopsies for the histopathologic grade with regard to the surgical resection might also arise from tumour heterogeneity with inter- and intratumoral variations of the histopathologic grade.[5,6] As such, co-localized biopsies might yield a better concordance of the histopathologic grade than reported in **Chapter 2** and better than nephroureterectomies when tumours are only assessed partly. Still, it is tenable that the histopathology of surgical resections may be a superior reference standard for diagnostic accuracy studies. As described in this thesis, nephroureterectomies introduce selection bias. The selection bias arises from the risk-stratification for treatment selection, resulting in bias towards high-grade UTUC with potentially more aggressive tumour biology than a cohort in which ureterorenoscopic biopsies are used as the reference standard. However, the exact implications of the two reference standards are difficult to define. Ultimately, the choice of reference standard is also influenced by the low prevalence of UTUC in the absence of multicentre studies. In comparison to ureterorenoscopic biopsies, the use of surgical resections as the reference standard limits the size of the study cohort within the same timeframe. In future studies, the reference standard will remain a point of discussion. A combination of diagnostic tests could possibly shine new light on this debate.

In conclusion, the present diagnostic approach to UTUC and its risk-stratification for treatment selection are not ideal. OCT and CLE hold the potential to improve the current diagnostic approach. By overcoming these diagnostic challenges, we will be able to deliver timely and individualized treatments for UTUC with less over- and under-treatment. Furthermore, FISH holds the potential to function as a triage test to reduce the number of follow-up ureterorenoscopies following kidney-sparing treatment. The findings of this thesis justify future efforts to advance the state of development of OCT, CLE and FISH for the diagnosis and grading of UTUC. The future looks bright with optical biopsies.

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APPENDIX

Appendix

English Summary

List of abbreviations

CLE – confocal laser endomicroscopy
FISH – fluorescence in situ hybridization
μ_{OCT} – optical coherence tomography attenuation coefficient
OCT – optical coherence tomography
UTUC – upper tract urothelial carcinoma

Seeing upper tract urothelial carcinoma in a new light

This thesis provides a collection of studies that explored the shortcomings of the current diagnostic approach to upper tract urothelial carcinoma (UTUC) and that investigated the use of novel optical techniques for UTUC diagnosis.

In **Chapter 1**, the background of upper tract urothelial carcinoma is introduced, touching upon the challenge to distinguish reliably and accurately between low-grade and high-grade UTUC with ureterorenoscopic biopsies. It follows that some patients, who qualify for kidney-sparing treatment according to one of the criteria recommended for the disease specific risk-stratification, might be stratified incorrectly. As a result, we should seek new ways to distinguish between low-grade and high-grade UTUC to warrant optimal oncologic outcomes.

Emerging optical imaging modalities such as optical coherence tomography (OCT) and confocal laser endomicroscopy (CLE) may hold the potential to overcome the diagnostic challenges at hand. Only by overcoming these diagnostic challenges, we will be able to deliver effective single modality treatments to some patients, offer others safe renal-sparing ureterorenoscopic treatment with ureterorenoscopic follow-up, and potentially define a third subset of patients with features beyond histopathologic grading and staging that may benefit from multimodality therapy.

Chapter 2 provides the first nationwide and currently largest study to address the diagnostic yield and the concordance of ureterorenoscopic biopsies for UTUC diagnosis and grading. The study was based on excerpts of the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA) from 2011 until 2018. We found that one out of ten ureterorenoscopic biopsies is non-diagnostic. This means that the biopsy yields insufficient tissue or tissue of insufficient quality for a reliable histopathologic diagnosis. Meanwhile, in case of UTUC diagnosis, the diagnostic yield of ureterorenoscopic biopsies for histopathologic grading was high. Yet, one third of the biopsy-based low-grade UTUC was upgraded to a high histologic grade in the surgical resection. It follows that one third of patients, who qualify for kidney-sparing treatment according to one of the criteria recommended by the risk-stratification of the EAU guidelines, might be stratified incorrectly. Our findings, therefore, stress the need for improvements in the diagnostic paradigm.

Chapter 3 presents the first study that assessed the diagnostic accuracy, inter-rater and intra-rater agreement of grade predictions based on the gross visual appearance of papillary UTUC during digital ureterorenoscopy. Despite

acceptable intra-rater agreement, the findings of this study illustrate low inter-rater agreement and unacceptable accuracies of grade predictions for low-grade and high-grade papillary UTUC. Consequently, one can conclude that grade predictions based on the ureterorenoscopic appearance of papillary UTUC should be used carefully or not in the diagnostic paradigm of UTUC.

Chapter 4 provides a comprehensive review of the literature, assessing the current state of development of OCT for application in bladder, upper urinary tract, kidney, prostate, testis, and penile tumours. Overall, the current state of development suggests that OCT holds the diagnostic potential to serve as an add-on or replacement test for in-vivo diagnostics of bladder cancer, upper urinary tract cancer, kidney cancer and penile cancer. So far, the current state of development has reached an exploratory stage. Further technical refinements of OCT are necessary to meet all clinical requirements and to maximize the potential gain in diagnostic value.

Chapter 5 presents a post-hoc analysis of in-vivo ureterorenoscopic OCT images that investigated a cut-off value for the attenuation coefficient for OCT-based grading of UTUC. As previously described by Bus et al, the decay of the OCT signal with tissue depth (the attenuation coefficient, μ_{OCT}) enables the differentiation between low-grade and high-grade UTUC. The present study provides an improved μ_{OCT} cut-off value for UTUC grading with quantitative assessment of ureterorenoscopic OCT images owing to a larger study population and the improved in-house developed software for μ_{OCT} analysis. Validation of the cut-off value is required to confirm the diagnostic potential of quantitative OCT assessment for UTUC grading.

Chapter 6 showcases the study protocols of two explorative studies that investigated the diagnostic ability of in-vivo CLE for diagnosis and grading of urothelial carcinoma in the bladder and the upper urinary tract. Both study protocols lead to the study discussed in Chapter 7 and the study of Liem et al. Additionally, Chapter 6 provides a link to a peer-reviewed video, in which the cystoscopic and ureterorenoscopic application of CLE imaging for the diagnosis of urothelial carcinoma is described.

As mentioned above, the findings of the explorative study of in-vivo CLE for the diagnostics of UTUC are described in Chapter 7. In this study, the prevalence and the diagnostic value of the previously proposed CLE criteria for low-grade and high-grade UTUC were investigated. We identified that the assessment of in-vivo CLE images with the proposed CLE criteria enables accurate grading of papillary UTUC. The diagnostic yield, however, is suboptimal and requires refinements. Based on the most prevalent CLE features, being ‘cellular organ-

ization', 'cellular morphology' and 'cohesiveness of cells', a simplified scoring system was proposed to aid the quantitative assessment for CLE-based grading of UTUC. Future studies should validate the proposed scoring system to confirm the diagnostic potential of qualitative CLE assessment for UTUC grading.

Chapter 8 presents a feasibility study, in which the use of fluorescence in situ hybridization (FISH, UroVysion®) was investigated for the detection of UTUC in 1 mL of selectively collected upper urinary tract urine. We found that FISH was feasible in all 1 mL samples with an estimated sensitivity and specificity for UTUC diagnosis of 90% and 80%, respectively. Subsequently, from a technical point of view, one may conclude that FISH analysis in small amounts of selectively collected upper tract urine could serve as an outpatient triage test to assess the necessity of follow-up ureterorenoscopy. Implementation of a triage test would lead to a reduction in the number of ureterorenoscopies and consequently reduce the burden of ureterorenoscopic procedures in the follow-up after kidney-sparing surgery. Further studies are, however, required to assess the diagnostic accuracy of FISH for the proposed application.

In **Chapter 9**, the findings of this thesis are discussed. In short, the findings highlight the shortcomings of the diagnostic approach to UTUC, and provide stepping-stones towards the implementation of optical imaging techniques to overcome these shortcomings in the near future.

Appendix

Dutch Summary | Samenvatting

Lijst met afkortingen
CLE – confocale laser endomicroscopie
FISH – fluorescentie in situ hybridisatie
OCT – optische coherentie tomografie

Urotheelkanker in de hoge urinewegen in een nieuw licht

Dit proefschrift bestaat uit wetenschappelijke onderzoeken die de tekortkomingen van de huidige diagnostiek voor urotheelkanker in de hoge urinewegen in kaart brengen. Deze tekortkomingen kunnen worden aangepakt door nieuwe optische technieken toe te passen. Onderzoeken naar drie van deze optische technieken worden uitgelicht in dit proefschrift.

Hoofdstuk 1 biedt achtergrondinformatie over urotheelkanker in de hoge urinewegen. Deze vorm van kanker ontstaat uit het slijmvlies dat de binnenzijde van de urineleiders en de nierbekkens bekleedt. De urineleiders en de nierbekkens vormen samen de hoge urinewegen. Bij een verdenking op kanker in de hoge urinewegen kunnen door middel van een ureterorenoscopie (een kijkoperatie van de hoge urinewegen) de urinewegen visueel worden beoordeeld. Bovendien kunnen tijdens een ureterorenoscopie biopten (weefselhapjes) van het verdachte weefsel worden genomen. De biopten worden aansluitend door een patholoog beoordeeld om een diagnose te stellen. Indien het urotheelkanker betreft, dan wordt er aan de hand van de biopten ook de agressiviteit van de kanker bepaald. De agressiviteit van de kanker wordt weergegeven als histopathologische gradering (laaggradige versus hooggradige kanker) waarbij een hooggradige kanker een slechtere prognose kent.

Hooggradig urotheelkanker dient over het algemeen te worden behandeld middels een ‘radicale nefro-ureterectomie’. Bij een ‘radicale nefro-ureterectomie’ wordt de gehele nier, urineleider en blaasmanchet aan de aangedane kant verwijderd. Het verwijderen van deze orgaanstructuren kan leiden tot nierfalen met mogelijk dialyse tot gevolg. In tegenstelling tot hooggradig urotheelkanker kan laaggradig urotheelkanker in sommige gevallen ook middels een niersparende therapie worden behandeld. Bij laaggradig urotheelkanker in de urineleider kan gekozen worden om slechts het aangedane stuk urineleider chirurgisch te verwijderen waardoor de nier aan de aangedane kant behouden blijft. Daarnaast kan laaggradig urotheelkanker tijdens een kijkoperatie met een laser worden weggebrand. Deze laatstgenoemde niersparende laserbehandeling wordt steeds populairder, echter een belangrijke voorwaarde hiervoor is dat het zeker een laaggradige kanker betreft.

Een accurate bepaling van de agressiviteit van de kanker is dus essentieel voor het kiezen van de beste behandeling. De nauwe anatomie en de ligging van de hoge urinewegen bemoeilijken het verrichten van betrouwbare diagnostiek. De ureterorenoscopische biopten leveren niet altijd voldoende weefsel op om de kanker te graderen (beperkte diagnostische opbrengst). Bovendien lijkt de gradering aan de hand van de biopten niet altijd accuraat (beperkte

diagnostische accuratesse). Dit leidt mogelijk tot suboptimale behandelkeuzes. Het doel van dit proefschrift is derhalve om de tekortkomingen van de huidige diagnostische aanpak van urotheelkanker in de hoge urinewegen te onderzoeken. Bovendien worden in dit proefschrift veelbelovende optische technieken onderzocht die het diagnostisch proces voor urotheelkanker in de hoge urinewegen in de toekomst kunnen verbeteren. Deze optische technieken betreffen optische coherentie tomografie (OCT), confocale laser endomicroscopie (CLE) en fluorescentie in situ hybridisatie (FISH).

In **hoofdstuk 2** wordt de eerste landelijke en momenteel grootste studie naar de diagnostische opbrengst en de diagnostische accuratesse van ureterorenoscopische biopten voor de diagnose en gradering van urotheelkanker in de hoge urinewegen beschreven. De studie is gebaseerd op uittreksels uit het ‘Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief’ (PALGA) van 2011 tot 2018. In deze studie blijkt dat één op de tien ureterorenoscopische biopten niet-diagnostisch is. Dit betekent dat de biopsie onvoldoende weefsel of weefsel van onvoldoende kwaliteit oplevert om een betrouwbare histopathologische diagnose te kunnen stellen. In het geval van urotheelkanker is de diagnostische opbrengst van de biopten voor de kanker gradering hoog. Desondanks blijkt een derde van de op de biopsie gebaseerde laaggradige urotheelkanker uiteindelijk toch hooggradig te zijn in de chirurgische resectie. Dit betekent dat een derde van de patiënten, die in aanmerking kwamen voor een niersparende behandeling volgens de richtlijnen van de ‘European Association of Urology’, mogelijk verkeerd worden behandeld. De bevindingen van dit onderzoek benadrukken dan ook de noodzaak van verbeteringen in het diagnostisch proces.

Hoofdstuk 3 beschrijft de eerste studie naar de diagnostische accuratesse, intra- en inter-observer overeenkomsten voor de voorspelling van de histopathologische gradering van urotheelkanker in de hoge urinewegen aan de hand van visuele kenmerken tijdens digitale ureterorenoscopie. Ondanks een acceptabele intra-observer overeenkomst is de inter-observer overeenkomst laag. Bovendien is de diagnostische accuratesse voor de voorspelling van de histopathologische gradering zeer laag. Dit betekent dat de ureterorenoscopische kenmerken van urotheelkanker in de hoge urinewegen niet geschikt zijn om de histopathologische gradering te voorspellen. In het diagnostisch proces voor urotheelkanker in de hoge urinewegen is dus een zeer beperkte dan wel geen plaats voor een dergelijke voorspelling van de histopathologische gradering. Deze bevindingen benadrukken de noodzaak van verbeteringen in het diagnostisch proces om tijdens de ureterorenoscopie de histopathologische gradering accuraat te kunnen voorspellen. Een accurate beoordeling van de histopathologische gradering gedurende de diagnostische ureterorenoscopie

is essentieel om nog gedurende dezelfde ingreep een optimale behandelkeuze te kunnen maken.

Hoofdstuk 4 geeft een overzicht van gepubliceerde studies naar de toepassing van OCT bij nier-, prostaat-, testis-, penis-, en urotheelkanker in de blaas en hoge urinewegen. OCT is het optische equivalent van echografie. In plaats van geluid wordt laserlicht het weefsel ingestuurd. Het gereflecteerde licht wordt gemeten en omgezet in een beeld van het beoordeelde weefsel. Uit de huidige literatuur blijkt dat OCT de diagnostische potentie heeft om als aanvullende of als vervangende test voor de standaard weefselbiopten voor de diagnostiek van nier-, penis- en urotheelkanker zou kunnen dienen. Tot nu toe bevindt zich de huidige stand van ontwikkeling echter slechts in een verkennend stadium. Grotere studies zijn nodig om de diagnostische waarde verder in kaart te brengen en te verifiëren. Bovendien zijn technische verfijningen nodig om de diagnostische waarde te maximaliseren.

In **hoofdstuk 5** wordt een verbeterde absorptiecoëfficiënt afkapwaarde van ureterorenoscopische OCT-beelden voor de gradering van urotheelkanker in de hoge urinewegen gepresenteerd. De huidige studie omvat de tot nu toe grootste onderzoekspopulatie en een verbeterde software voor het berekenen van de absorptiecoëfficiënt. De absorptiecoëfficiënt is de mate van verstrooiing van het optische signaal met de penetratiediepte van het weefsel. Zoals eerder beschreven door Bus et al, kan aan de hand van de absorptiecoëfficiënt onderscheid gemaakt worden tussen laaggradige en hooggradige urotheeltumoren. In de huidige studie van dit hoofdstuk werd een nieuwe afkapwaarde voor de kwantitatieve beoordeling van OCT-beelden verkregen om urotheelkanker in de hoge urinewegen beter te kunnen graderen met OCT. Validatie van de nieuwe afkapwaarde is in een toekomstige studie nodig om de diagnostische accuratesse te bevestigen.

Hoofdstuk 6 toont het studieprotocol van twee CLE-studies. In deze studies wordt het onderscheidend vermogen van in-vivo CLE voor de diagnose en gradering van urotheelkanker in de blaas en de hoge urinewegen onderzocht. CLE is een laserlicht techniek waarbij het licht gefocust wordt in een dun vlak, waardoor er beelden van het weefsel op microscopisch niveau worden gegenereerd. De microscopische beelden zijn digitaal en worden in realtime gegenereerd. Voor het maken van CLE-beelden is het nemen van een weefselbiopt niet meer nodig. De studieprotocollen hebben geleid tot de studie in hoofdstuk 7 en een studie van Liem et al. Daarnaast is in hoofdstuk 6 een link naar een ‘peer-reviewed’ video opgenomen. In deze video wordt de toepassing van CLE tijdens cystoscopie (kijkonderzoek van de blaas) en ureterorenoscopie (kijkonderzoek van de hoge urinewegen) uitgelegd.

In **hoofdstuk 7** worden de bevindingen van de studie naar in-vivo CLE voor de diagnostiek van urotheelkanker in de hoge urinewegen beschreven. Deze studie bevestigt dat de reeds gepubliceerde CLE-criteria gebruikt kunnen worden voor gradering van urotheelkanker in de hoge urinewegen met een acceptabele diagnostische accuratesse. De diagnostische opbrengst van CLE is echter suboptimaal en vereist verfijning van de techniek. Op basis van de meest voorkomende CLE-criteria werd tevens een vereenvoudigd scoresysteem uitgewerkt. Het doel van dit nieuwe scoringssysteem is het vereenvoudigen van de kwantitatieve beoordeling van CLE-beelden voor de gradering van urotheelkanker. Validatie van dit scoresysteem is nodig om de diagnostische waarde hiervan te bevestigen.

Hoofdstuk 8 beschrijft een studie waarbij gebruik wordt gemaakt van FISH voor de detectie van urotheelkanker in de hoge urinewegen. FISH is een techniek waarbij voor urotheelkanker typische veranderingen van de chromosomen worden gemarkeerd en middels fluorescentiemicroscopie gevisualiseerd, zodat urotheelkankercellen visueel gedetecteerd kunnen worden. Dit is de eerste studie waarbij wordt onderzocht of het haalbaar is om urotheelkanker te detecteren in 1 ml urine uit de hoge urinewegen. Uit de studieresultaten blijkt dat het mogelijk is om urotheelkanker van de hoge urinewegen in een zeer beperkte hoeveelheid urine met FISH op te sporen. Vanuit een technisch oogpunt is het derhalve mogelijk om de onderzochte methode als een triagetest tijdens cystoscopieën op de polikliniek toe te passen om daarmee de noodzaak van een follow-up ureterorenoscopie, na eerdere laserbehandeling van urotheelkanker van de hoge urinewegen, te beoordelen. Een triagetest zou het aantal follow-up ureterorenoscopieën kunnen beperken. Minder follow-up ureterorenoscopieën resulteert in een minder belastend vervolgtraject voor de patiënt. Tevens zou dit ook leiden tot een verminderde belasting van de operatiecapaciteit. FISH blijkt een hoge geschatte diagnostische accuratesse te kennen maar nader onderzoek is nodig naar de diagnostische accuratesse van FISH en de voorgestelde toepasbaarheid op de polikliniek in kaart te brengen.

In **hoofdstuk 9** worden de bevindingen van dit proefschrift bediscussieerd. De bevindingen benadrukken dat er ruimte is voor verbetering van de huidige diagnostische aanpak van urotheelkanker in de hoge urinewegen. Optische technieken zoals OCT, CLE en FISH hebben de potentie om de tekortkomingen te verhelpen. De uitkomsten van de huidige studies naar deze optische technieken voor de diagnostiek van urotheelkanker in de hoge urinewegen bieden een opstap naar verbeteringen in de diagnostische aanpak en daarmee mogelijk ook verbeteringen van de oncologische behandeluitkomsten. De beste behandeling begint immers met de beste diagnose. Verder onderzoek blijft echter hard nodig voordat optische biopten kunnen worden geïmplementeerd in de reguliere zorg.

Appendix

German Summary | Zusammenfassung

Liste mit Abkürzungen

CLE – konfokale Laserendomikroskopie
FISH – Fluoreszenz-in-Situ-Hybridisierung
OCT – optische Kohärenztomographie

Urothelkarzinom der oberen ableitenden Harnwege in einem neuen Licht

Diese Promotionsarbeit (Ph.D.) besteht aus mehreren wissenschaftlichen Abhandlungen, die einerseits die Unzulänglichkeiten der gegenwärtigen Diagnostik des Urothelkarzinoms der oberen ableitenden Harnwege darstellt und andererseits neue vielversprechende optische bildgebende Techniken zur erweiterten Diagnostik des Urothelkarzinoms untersucht. Diese Promotionsarbeit liefert somit einen Beitrag die bestehenden Unzulänglichkeiten der Diagnostik des Urothelkarzinoms zu verbessern.

Kapitel 1 ist eine Einführung in das Thema Urothelkarzinom der oberen ableitenden Harnwege. Das Urothelkarzinom entsteht in der Schleimhaut, die die Innenseite der Harnleiter und der Nierenbecken auskleidet. Die Harnleiter und die Nierenbecken bilden die sogenannten oberen ableitenden Harnwege. Bei einem Verdacht eines Karzinoms der oberen ableitenden Harnwege werden mittels einer Ureterorenoskopie (Spiegelung der oberen ableitenden Harnwege) die Harnleiter und die Nierenbecken visuell beurteilt. Außerdem können während einer Ureterorenoskopie Biopsien (Gewebeproben) von verdächtig erscheinenden Strukturen entnommen werden. Die anschließende Beurteilung der Gewebeproben durch den Pathologen führt zur endgültigen Diagnose. Sofern ein Urothelkarzinom vorliegt wird anhand dieser Gewebeproben auch die Aggressivität (Malignität) und Invasivität des Karzinoms bestimmt. Die Malignität des Karzinoms wird mittels einer histopathologischen Schweregradeinteilung (niedrig- bzw. hochmaligne) widergegeben. Die hochmaligne Form des Karzinoms hat eine ungünstigere Prognose.

Hochmaligne Urothelkarzinome müssen in aller Regel durch eine „radikale Nephro-Ureterektomie“ behandelt werden. Bei einer „radikalen Nephro-Ureterektomie“ wird die gesamte Niere und der dazu gehörende Harnleiter entfernt. Das Entfernen dieser Organstrukturen kann eine Niereninsuffizienz mit der Notwendigkeit der Dialyse zur Folge haben. Im Gegensatz zu hochmalignen Urothelkarzinomen kann das niedrigmaligne Urothelkarzinom in manchen Fällen durch eine Nieren-erhaltende Therapie behandelt werden. Bei einem niedrigmalignen Urothelkarzinom des Harnleiters ist z.B. eine mögliche Behandeloption lediglich den betroffenen Anteil des Harnleiters chirurgisch zu entfernen. Die betreffende Niere und ihr Harnleiter werden dadurch erhalten. Auch besteht bei einem niedrigmalignen Urothelkarzinom die Option das Karzinom mittels Lasertherapie ureterorenoskopisch zu entfernen. Diese Nieren-erhaltende Lasertherapie nimmt an Popularität zu. Diese ist aber nur dann sicher, wenn es sich tatsächlich nur um ein niedrigmalignes Urothelkarzinom handelt. Eine akkurate Bestimmung der Malignität des Karzinoms ist somit essentiell, um die adäquate Behandlungsmethode auswählen zu können.

Die Anatomie und die Lage der oberen ableitenden Harnwege erschweren die Durchführung einer zuverlässigen Diagnostik. Die ureterorenoskopisch gewonnenen Biopsien liefern nicht immer ausreichendes Gewebematerial, um eine Schweregradeinteilung in niedrig- bzw. hochmaligne durchführen zu können, d.h. es findet sich eingeschränkte diagnostische Ergiebigkeit des gewonnenen Materials. Außerdem scheint die Schweregradeinteilung durch die histopathologische Begutachtung der Biopsien - selbst mit ausreichendem Gewebematerial - nicht immer korrekt zu sein. D.h. es findet sich zudem das Problem der eingeschränkten diagnostischen Genauigkeit. In der Summe kann dies zu suboptimalen Behandlungsstrategien führen.

Das übergeordnete Ziel dieser Promotionsarbeit ist deshalb einerseits diese Unzulänglichkeiten des derzeitigen diagnostischen Vorgehens und seinen Möglichkeiten bei Urothelkarzinomen der oberen ableitenden Harnwege zu untersuchen. Andererseits werden in dieser Promotionsarbeit erfolgversprechende optische Techniken untersucht, die den Prozess der Diagnostik des Urothelkarzinoms der oberen ableitenden Harnwege in der Zukunft verbessern werden. Die verwendeten optischen Techniken sind die optische Kohärenztomographie (OCT), die konfokale Laserendomikroskopie (CLE) und die Fluoreszenz-in-Situ-Hybridisierung (FISH).

In **Kapitel 2** wird die erste nationale und derzeit weltweit größte Studie hinsichtlich Ergiebigkeit und Genauigkeit der Diagnostik von ureterorenoskopisch gewonnenem Biopsiematerial zur Diagnose und Schweregradeinteilung von Urothelkarzinom der oberen ableitenden Harnwege vorgestellt. Die Studie basiert auf der Auswertung des niederländischen „Pathologisch-anatomisch nationalen Archiv“ (PALGA) von 2011 bis 2018. Diese Studie zeigt auf, dass eine von zehn ureterorenoskopischen Biopsien nicht beurteilbar war. Das bedeutet, dass die Biopsie unzureichendes Material bzw. Material mit unzureichender Qualität lieferte, um eine Diagnose histo-pathologisch zuverlässig stellen zu können. Bei Nachweis eines Urothelkarzinoms wurde bei beinah allen dieser Biopsien der Schweregrad ermittelt. Nichtsdestoweniger wurde ein Drittel der Biopsie-basierten niedrigmalignen Urothelkarzinome nach histo-pathologischer Beurteilung des dann chirurgisch resezierten Präparats nach oben angepasst (hochmaligne statt niedrigmaligne). Das bedeutet, dass ein Drittel der Patienten, auf Basis der histo-pathologischen Beurteilung des durch Biopsie gewonnen Materials möglicherweise untertherapiert werden. Unsere Ergebnisse unterstreichen dann auch deutlich die Notwendigkeit, dass der Prozess der Diagnostik des Urothelkarzinoms der oberen ableitenden Harnwege verbessert werden muss.

Kapitel 3 ist die erste Studie, die die Genauigkeit der Diagnostik, die intra- und interobserver Übereinstimmungen bei der Vorhersage bzw. Festlegung des histopathologischen Schweregrad bei Urothelkarzinom in den Harnleitern an Hand visueller Merkmale im Rahmen der Ureterorenoskopie untersucht. Trotz einer akzeptablen intraobserver Übereinstimmung ist die interobserver Übereinstimmung niedrig. Zudem ist auch die Genauigkeit von Ureterorenoskopie-basierten Diagnosen für die Vorhersage der histopathologischen Schweregradeinteilung unakzeptabel niedrig. Das bedeutet, dass die ureterorenoskopisch sichtbaren Merkmale des Urothelkarzinom in den oberen ableitenden Harnwegen nicht ausreichend sind, um eine histopathologisch ermittelte Schweregradeinteilung vorhersagen zu können. Die vorliegenden Untersuchungsergebnisse zeigen auf, dass die Notwendigkeit besteht, dass der Diagnostik-Prozess deutlich zu verbessern ist, um während der Ureterorenoskopie in der Lage zu sein eine histo-pathologische Schweregradeinteilung durchführen zu können. Dies sollte dann einen unmittelbaren Effekt auf eine dann optimierte Behandlungsstrategie haben.

Kapitel 4 gibt eine Übersicht der publizierten Studien zur Anwendung der OCT bei Urothelkarzinomen der Harnblase und der oberen ableitenden Harnwege, sowohl als auch bei Nieren-, Prostata-, Testis- und Peniskarzinomen. OCT ist das optische Äquivalent der Sonographie. Statt Ultraschallwellen werden Laserstrahlen in das Gewebe gesendet. Das reflektierte Licht wird gemessen und in ein Bild des zu beurteilenden Gewebes umgesetzt. Daten der bestehenden Literatur geben Anlass anzunehmen, dass OCT ein erhebliches diagnostisches Potential hat, um zusätzliche relevante Informationen zu liefern oder gar die Standard-Gewebebiopsien zur Diagnostik von Urothelkarzinomen, Nieren- und Peniskarzinomen in Zukunft ersetzen zu können. Bislang ist die aktuelle Entwicklung allerdings noch in einem Beginnstadium. Größere Studien sind notwendig, um das diagnostische Potential zu verifizieren. Außerdem sind technische Verbesserungen nötig, um alle klinischen Anforderungen zu erfüllen und den Gewinn des diagnostischen Wertes der OCT zu maximieren.

In **Kapitel 5** wird ein verbesserter Grenzwert für den Absorptionskoeffizienten (Dämpfungskonstante) der ureterorenoskopischen OCT-Bilder für die Schweregradeinteilung des Urothelkarzinom der oberen ableitenden Harnwege präsentiert. Die vorliegende Studie umfasst die größte bislang untersuchte Patientenpopulation. Es wurde eine optimierte Software für die Berechnung des Absorptionskoeffizienten benutzt. Der Absorptionskoeffizient ist ein Maß für die Streuung des optischen Signals in Abhängigkeit von der Penetrationstiefe des untersuchten Gewebes. Wie bereits von Bus et al beschrieben kann an Hand des Absorptionskoeffizienten zwischen niedrig- und hochgradig malignem Urothelkarzinom unterschieden werden. In der vorliegenden Studie wird ein

verbesserter Grenzwert für die quantitative Beurteilung der OCT-Bilder hinsichtlich der Schweregradeinteilung des Urothelkarzinoms der oberen ableitenden Harnwege vorgestellt. Die Validierung des neu ermittelten Grenznwert ist allerdings notwendig, um die vielversprechende diagnostische Genauigkeit zu bestätigen.

Kapitel 6 demonstriert das Studienprotokoll von zwei der hier vorgelegten Studien, die das Vermögen der in-vivo CLE-Diagnostik für die Diagnose und Schweregradeinteilung des Urothelkarzinom der Harnblase und der oberen ableitenden Harnwege untersucht. CLE ist eine Laserlichttechnik wobei das Laserlicht auf eine sehr dünne Fläche fokussiert wird. Somit werden Bilder des Gewebes auf mikroskopischem Niveau generiert, ohne dass hierfür eine Lichtmikroskopie von Biopsiematerial notwendig wird. Das Studienprotokoll war Voraussetzung für die in Kapitel 7 aufgeführte Studie und die Studie von Liem et al. Zudem beinhaltet Kapitel 6 einen Link zu einer „peer-reviewed" Video-Studie, in der die Anwendung von CLE im Rahmen einer Zystoskopie (Harnblasenspiegelung) und Ureterorenoskopie dargelegt wird.

In **Kapitel 7** werden - wie bereits in Kapitel 6 angekündigt - die Ergebnisse der Studie der in-vivo CLE-Diagnostik von Urothelkarzinomen der oberen ableitenden Harnwege beschrieben. Diese Studie bestätigt, dass die bereits publizierten CLE-Kriterien für die Schweregradeinteilung von Urothelkarzinomen in den oberen ableitenden Harnwegen mit einer akzeptablen diagnostischen Übereinstimmung gebraucht werden können. Die diagnostische Ergiebigkeit von CLE ist allerdings noch suboptimal und erfordert eine Verbesserung der Technik. Auf Basis der am meisten vorkommenden CLE-Kriterien wird ein Scoring-System erarbeitet, um die quantitative Beurteilung von CLE-Bildern für die Schweregradeinteilung von Urothelkarzinomen zu vereinfachen. Die Validierung des neuen Scoring-Systems ist in der Folge notwendig, um seinen diagnostischen Wert zu bestätigen.

In **Kapitel 8** wird die erste Machbarkeitsstudie der FISH-Diagnostik in einem Milliliter Urin aus den oberen ableitenden Harnwegen zur Detektion des Urothelkarzinom präsentiert. FISH ist eine bekannte Technik, in der Urothelkarzinom-typische Veränderungen auf chromosomalem Niveau markiert und mittels der Fluoreszenzmikroskopie sichtbar gemacht werden können. Die vorliegende Studie zeigt, dass es möglich ist das Vorliegen eines Urothelkarzinom der oberen ableitenden Harnwege in einer sehr geringen Urinmenge mittels FISH nachzuweisen. Die beschriebene Technik ermöglicht prinzipiell die Anwendung der vorgestellten Methode als Triagetest während ambulant durchzuführender Zystoskopien. Die Absicht ist die Notwendigkeit weiterer

Verlaufsureterorenoskopien nach vorhergehender Laserbehandlung von Urothelkarzinomen der oberen ableitenden Harnwege festzulegen. Ein solcher Triagetest könnte die Anzahl der bislang notwendigen Verlaufs-ureterorenoskopien verringern und somit zur Verminderung der Belastung des Patienten beitragen. Weitere Untersuchungen sind allerdings notwendig, um die diagnostische Genauigkeit für die geschilderte Anwendung in der ambulanten Patientenbetreuung zu überprüfen.

In **Kapitel 9** werden die Ergebnisse dieser Promotionsarbeit diskutiert. Die Ergebnisse bestärken die Notwendigkeit die gegenwärtige Diagnostik des Urothelkarzinoms der oberen ableitenden Harnwege zu erweitern und bestätigen gleichzeitig die Möglichkeiten der untersuchten Methoden. Optische Techniken wie OCT, CLE und FISH haben das Potential die derzeitig noch bestehenden Beschränkungen zu verbessern. Die Ergebnisse der hier präsentierten Studien hinsichtlich der Verwendung der genannten bildgebenden Techniken zur Diagnostik des Urothelkarzinoms der oberen ableitenden Harn-wege zeigen einen Zuwachs an Verbesserungen in der Diagnostik und sollten dadurch eine Verbesserung der Behandlungserfolge ermöglichen. Unverändert gilt, dass die beste Behandlung mit einer optimalen Diagnostik beginnt, die eine präzise Diagnose ermöglicht. Weitere wissenschaftliche Untersuchungen sind allerdings notwendig bevor optische Methoden in die Routinediagnostik und Behandlung implementiert werden können, um möglicherweise auf Biopsien zu verzichten.

Appendix

Author Contributions

Author Contributions

Legends:

Study concept and design:	1
Data acquisition:	2
Analysis and data interpretation:	3
Drafting of the manuscript:	4
Critical revision of the manuscript:	5
Study supervision:	6

Chapter 2
The Diagnostic Yield and Concordance of Ureterorenoscopic Biopsies for Grading of Upper Tract Urothelial Carcinoma: A Dutch Nationwide Analysis

J.E. Freund	1, 2, 3, 4, 6
M.J.C. Duivenvoorden	2, 3, 5
B.T. Sikma	2, 3, 5
R.W.M. Vernooij	3, 5
C.D. Savci-Heijink	1, 5
J.D. Legemate	2, 3, 5
T.M. de Reijke	1, 5, 6

Chapter 3
Upper Tract Urothelial Carcinoma Grade Prediction based on the ureteroscopic Appearance: Caution should be taken

J.E. Freund	1, 2, 3, 4
J.D. Legemate	2, 3, 5
J. Baard	2, 5
K. Saeb-Parsy	2, 5
O. Wiseman	2, 5
S. Doizi	2, 5
E. Emiliani	2, 5
A. Breda	2, 5
B.J. Boodt	2, 5
E.P. van Haarst	2, 5
M.M.G. Leeflang	3, 5
G.M. Kamphuis	1, 2, 3, 5

Chapter 4
Optical Coherence Tomography in urologic oncology: A comprehensive review

J.E. Freund	1, 2, 3, 4
M. Buijs	2, 3, 4
C.D. Savci- Heijink	1, 5
D.M. de Bruin	2, 3, 5
J.J.M.C.H. de la Rosette	1, 5
T.G. van Leeuwen	1, 5, 6
M.P. Laguna	1, 5, 6

Chapter 5
Grading Upper Tract Urothelial Carcinoma with the Attenuation Coefficient of in-vivo Optical Coherence Tomography

J.E. Freund	1, 2, 3, 4
D.J. Faber	3, 5
M.T. Bus	2, 5
T. G. van Leeuwen	1, 3, 5, 6
D.M. de Bruin	1, 3, 5, 6

Chapter 6
Confocal Laser Endomicroscopy for the Diagnosis of Urothelial Carcinoma in the Bladder and the Upper Urinary Tract: Protocols for Two Prospective Explorative Studies

J.E. Freund	1, 2, 3, 4
E.I.M.L. Liem	1, 2, 3, 4
J. Baard	1, 2, 5
D.M. de Bruin	1, 5
M.P. Laguna Pes	1, 5
C.D. Savci-Heijink	1, 5
T.G. van Leeuwen	1, 5
T.M. de Reijke	1, 5, 6
J.J.M.C.H. de la Rosette	1, 5, 6

Chapter 7
Confocal Laser Endomicroscopy for Upper Tract Urothelial Carcinoma: Validation of the Proposed Criteria and Proposal of a Scoring System for real-time Tumour Grading

J.E. Freund	1, 2, 3, 4
E.I.M.L. Liem	2, 3, 5
C.D. Savci-Heijink	2, 3, 5
J. Baard	2, 5
G.M. Kamphuis	2, 5
J.J.M.C.H. de la Rosette	1, 2, 5, 6
D.M. de Bruin	1, 3, 5, 6
T.G. van Leeuwen	5
T.M. de Reijke	5

Chapter 8
Fluorescence In Situ Hybridization in 1 mL Samples of selective Upper Tract Urine for the Detection of Upper Tract Urothelial Carcinoma: A Feasibility Study

J.E. Freund	1, 2, 3, 4
E.I.M.L. Liem	1, 2, 3, 5
C.D. Savci-Heijink	3, 5, 6
T.M. de Reijke	1, 5, 6

Appendix

List of Publications

List of publications

Part of this thesis

1. Freund JE, Duivenvoorden MJC, Sikma BT, et al. The Diagnostic Yield and Concordance of Ureterorenoscopic Biopsies for Grading of Upper Tract Urothelial Carcinoma: A Dutch Nationwide Analysis. J Endourol. 2020;34(9):907–13.
2. Freund JE, Legemate JD, Baard J, Saeb-Parsy K, Wiseman O, Doizi S, et al. Upper Tract Urothelial Carcinoma Grade Prediction Based on the Ureteroscopic Appearance: Caution Should be Taken. Urology. 2019;132:69–74.
3. Freund JE, Buijs M, Savci-Heijink CD, de Bruin DM, de la Rosette JJMCH, van Leeuwen TG, et al. Optical Coherence Tomography in Urologic Oncology: a Comprehensive Review. SN Compr Clin Med. 2019;1(2):67–84.
4. Freund JE, Faber DJ, Bus MT, van Leeuwen TG, de Bruin DM. Grading upper tract urothelial carcinoma with the attenuation coefficient of in-vivo optical coherence tomography. Lasers Surg Med. 2019;51(5):399–06.
5. Liem EI, Freund JE, Baard J, de Bruin DM, Laguna Pes MP, Savci-Heijink CD, et al. Confocal Laser Endomicroscopy for the Diagnosis of Urothelial Carcinoma in the Bladder and the Upper Urinary Tract: Protocols for Two Prospective Explorative Studies. JMIR Res Protoc. 2018;7(2):e34.
6. Freund JE, Liem EI, Baard J, Kamphuis G, Laguna MP, de Reijke TM, et al. Confocal Laser Endomicroscopy for the diagnosis of urothelial carcinoma in the bladder and the upper urinary tract. Videourology. 2018;32:4.
7. Freund JE, Liem EIML, Savci-Heijink CD, Baard J, Kamphuis GM, de la Rosette JJMCH, et al. Confocal laser endomicroscopy for upper tract urothelial carcinoma: validation of the proposed criteria and proposal of a scoring system for real-time tumor grading. World J Urol. 2019;37(10):155–64.
8. Freund JE, Liem EIML, Savci-Heijink CD, de Reijke TM. Fluorescence in situ hybridization in 1 mL of selective urine for the detection of upper tract urothelial carcinoma: a feasibility study. Med Oncol. 2019;36(1):10.

Other publications

9. Liem EIML, Freund JE, Savci-Heijink CD, De la Rosette JJMCH, Kamphuis GM, Baard J, et al. Validation of Confocal Laser Endomicroscopy Features of Bladder Cancer: The Next Step Towards Real-time Histologic Grading. Eur Urol Focus. 2020;6(1):81-87.
10. Lucas M, Liem EIML, Savci-Heijink CD, Freund JE, Marquering HA, van Leeuwen TG, et al. Toward Automated In Vivo Bladder Tumor Stratification Using Confocal Laser Endomicroscopy. J Endourol. 2019;33(11):930–7.
11. Legemate JD, Kamphuis GM, Freund JE, Baard J, Oussoren HW, Spijkerman IJB, et al. Pre-Use Ureteroscope Contamination after High Level Disinfection: Reprocessing Effectiveness and the Relation with Cumulative Ureteroscope Use. J Urol. 2019;201(6):1144–51.
12. Legemate JD, Zanetti SP, Freund JE, Baard J, de la Rosette JJMCH. Surgical teaching in urology: patient safety and educational value of 'LIVE' and 'SEMI-LIVE' surgical demonstrations. World J Urol. 2018;36(10):1673-9.
13. Legemate JD, Kamphuis GM, Freund JE, Baard J, Zanetti SP, Catellani M, et al. Durability of Flexible Ureteroscopes: A Prospective Evaluation of Longevity, the Factors that Affect it, and Damage Mechanisms. Eur Urol Focus. 2018;5(6):1105–11.
14. Baard J, Freund JE, de La Rosette JJMCH, Laguna MP. New technologies for upper tract urothelial carcinoma management. Curr Opin Urol. 2017;27(2):170–5.

Appendix

PhD Portfolio

General courses	ETCS	Year
Basic Microscopy Course 'In the footsteps of Antoni van Leeuwenhoek'	1.6	2018
Advanced Topics in Biostatistics	2.1	2017
Clinical Epidemiology: Evaluation of Medical Test	0.9	2017
How to write high impact papers and what to do when your manuscript is rejected	0.4	2017
Observational Clinical Epidemiology – Effects and Effectiveness	0.6	2016
The AMC World of Science	0.7	2016
Entrepreneurship in Health- and Life Science	1.5	2016
Searching for a Systematic Review	0.1	2016
Practical Biostatistics	1.1	2016
Clinical Epidemiology: Evaluation of Medical Tests	0.6	2016
Basiscursus Regelgeving en Organisatie voor Klinisch Onderzoekers (e-BROK)	1.0	2016
Evidence Based Searching	0.1	2016
Clinical Epidemiology 'Systematic Reviews'	0.7	2016

(Inter)national Conferences	Year
Symposium Beeldvorming in de Urologie V, Amsterdam, NL	2020
Annual Meeting of the Dutch Pathology Association, Arnhem, NL	2019
34th European Association of Urology Congress, Barcelona, Spain	2019
American Urology Association Annual Meeting, San Francisco, USA	2018
33rd European Association of Urology Congress, Copenhagen, DK	2018
Technology and Training in Endourology, Torino, Italy	2017
7th International Meeting on Challenges in Endourology, Paris, France	2017
Annual Meeting of the Dutch Association of Urology, Den Bosch, NL	2017
32nd European Association of Urology Congress, London, UK	2017
Global Congress on Bladder Cancer BLADDR, Brussels, Belgium	2016
6h International Meeting on Challenges in Endourology, Paris, France	2016

Presentations at International Conferences	Year
'Can we predict the histopathologic grade of upper tract urothelial carcinoma based on the ureteroscopic appearance?' – 34th European Association of Urology Congress, Barcelona, Spain	2019
'Assessing the proposed confocal laser endomicroscopy criteria for grading of upper tract urothelial carcinoma' – 34th European Association of Urology Congress, Barcelona, Spain	2019
'Optical Coherence Tomography for Grading of Upper Tract Urothelial Carcinoma: A first validation of the attenuation coefficient cut-off for low and high grade UTUC '- American Urology Association Annual Meeting 2018, San Francisco, USA	2018
'Optical biopsies for the diagnosis of upper tract urothelial carcinoma: How we do it' – 7th International Meeting on Challenges in Endourology, Paris, France	2017

Invited Talks / Faculty	Year
'OCT/CLE bij urinewegtumoren' - Symposium Beeldvorming in de Urologie V, Amsterdam, The Netherlands	2020
'Optical Computed Tomography for the diagnosis of UTUC '- Technology and Training in Endourology, Turino, Italy	2017

Teaching and supervising	Year
Daily supervision of a 3rd year medical student for the bachelor thesis titled 'The concordance of staging and stage prediction in the upper urinary tract'	2019
Daily supervision of a 3rd year medical student for the bachelor thesis titled 'The concordance of histopathological grading in the upper urinary tract'	2019
Daily supervision of a 3rd year medical student for the bachelor thesis titled 'Hoe veilig en effectief zijn antegrade spoelingen voor de behandeling van UTUC?'	2018
Giving scientific presentations and assisting live endourologic procedures during multiple international training courses and broadcasted masterclasses for urologists and urology residents at the Amsterdam University Medical Centers, location AMC.	2016 2017

Grants	Year
Grant (40 000 €) for the project "Optical biopsies for the diagnosis of urothelial carcinoma"	2017

Appendix

About the author

Jan Erik Freund was born on July 20th 1989 in Mainz, Germany. After having lived in various places in Germany, Jan Erik moved together with his parents and his sister to the Netherlands at the age of 14 years. During high-school, he joined the local rowing club in Hilversum, The Netherlands, where he picked up rowing and the Dutch language. In 2008, Jan Erik completed the International Baccalaureate at the International School Hilversum. In the same year, he enrolled in medical school at the University of Amsterdam. With the start of his medical studies, Jan Erik became an ambitious and dedicated rower at the student rowing club R.S.V.U. Okeanos. After becoming a Dutch citizen in 2009, he joined the Dutch national rowing team to compete at the World Rowing Championships for under 23 years in 2010 and 2011, and the 2013 Summer Universiade. During these years, Jan Erik and his teams became Dutch national champions. Following these accomplishments, Jan Erik decided to stop rowing to focus on his medical studies. He published his first research in the field of pediatric cardiology. With his dedication and enthusiasm, Jan Erik graduated from medical school with cum laude in 2016. During his medical internships, he had developed a special interest in Urology and diagnostics. This interest led him to pursue a position as a clinical PhD-candidate at the departments of Urology and Biomedical Engineering and Physics of the Amsterdam University Medical Centers, location AMC, University of Amsterdam. His PhD addressed various aspects of the diagnostics for upper tract urothelial carcinoma, including new optical imaging techniques for in-vivo histopathologic assessment. Despite his great interest in endourology, Jan Erik followed his passion for diagnostics. During his PhD, Jan Erik started his residency in Surgical Pathology in 2018. Currently, he is a resident at the department of Pathology at the Amsterdam University Medical Centers, location AMC. In the future, he aspires to continue research on optical imaging techniques and the use of artificial intelligence beyond image interpretation.

Appendix

Acknowledgement

I wish to express my gratitude to all the persons, who contributed to the completion of this thesis in various ways. I thank each one of them wholeheartedly, and I would like to recognize the commitment and support of some people in particular:

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Mara Buijs - Olivia Lodeizen - Jons van Hattum
Paul Bloemen - Lizzy Wijmans
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Pilar Laguna & Jean de la Rosette
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Colleagues at the department of Biomedical Engineering & Physics
Colleagues at the department of Pathology
All patients who participated in the studies at hand

Martje
Tine
Inga Marie
Mama & Papa

OCT
FISH
CLE