RECENT ADVANCES IN BLADDER CANCER DIAGNOSIS AND TREATMENT

EDITED BY: Jeremy Teoh and Daniele Castellani
PUBLISHED IN: Frontiers in Surgery

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88976-080-0
DOI 10.3389/978-2-88976-080-0

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RECENT ADVANCES IN BLADDER CANCER DIAGNOSIS AND TREATMENT

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Citation: Teoh, J., Castellani, D., eds. (2022). Recent Advances in Bladder Cancer Diagnosis and Treatment. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88976-080-0

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frontiers | Frontiers in Surgery

EDITORIAL
published: 12 April 2022
doi: 10.3389/fsurg.2022.890172

Editorial: Recent Advances in Bladder Cancer Diagnosis and Treatment

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Keywords: bladder cancer, urothelial carcinoma, urinary marker, lymphadenectomy, chemohyperthermia, BCG - bacille calmette-guérin vaccine, transurethral resection (TUR) of bladder

Editorial on the Research Topic

Recent Advances in Bladder Cancer Diagnosis and Treatment

According to GLOBOCAN data, bladder cancer is the 10th most common cancer worldwide, and there were 573,278 estimated new cases and 212,536 death worldwide in 2020 (1). Men are affected consistently higher than women with a male: female ratio ranging from 2:1 to 6:1 (2). The incidence of bladder cancer increases steeply after the age of 50 years, particularly due to exposures to risk factors such as cigarette smoking and occupational exposures in developed countries, and Schistosomiasis infection in Africa and the Middle East (3). Two third of bladder cancer cases are non-muscle-invasive at diagnosis and tend to recur during patient life.

The world population is expected to increase from the current 7.6 billion to 8.5 billion people in 2030, and this demographic change will have undoubtedly a huge influence on bladder cancer occurrence, prevalence, and mortality with a growing burden on clinical care (4). Despite various types of surgical and systemic treatments, the oncological outcomes of bladder cancer are still unsatisfactory.

Recently, there have been a lot of advances in the diagnosis and treatment of bladder cancer. Although cystoscopy is the gold standard in the primary diagnosis and surveillance of bladder cancer, urinary markers and enhanced imaging are likely to play an important role in the future. In addition, newer surgical approaches have been adopted in the management of bladder cancer. The main aim of this Research Topic was to cover promising, recent, and future research trends in the field of Bladder Cancer, with straightforward key messages for clinicians interested in this field. This special Research Topic was launched in January 2021 and closed in September 2021. Within 8 months, 15 manuscripts were submitted, of which 9 were accepted. There were 6 reviews and 3 original articles. By February 2022, this special Research Topic reached 14,374 views. The subjects covered were urine biomarkers (Chai et al.; Sugeeta et al.), en bloc resection of bladder tumor (Fankhauser et al.; Liu et al.), the adequacy of pelvic lymphadenectomy during radical cystectomy (Jena et al.), the role of two T1 sub staging systems on recurrence and progression (Asimakopoulos et al.), the role of macroscopic image enhancement in the diagnosis of non-muscle-invasive bladder cancer (Mulawkar et al.), the role of pathologists in handling and reporting bladder cancer samples (Mazzucchelli et al.), and intravesical chemohyperthermia vs. bacillus Calmette-Guerin instillation for intermediate- and high-risk non-muscle-invasive bladder (Zhao et al.).

Edited and reviewed by:
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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *05 March 2022*
Accepted: *24 March 2022*
Published: *12 April 2022*

Citation:
Teoh JY-C and Castellani D (2022) Editorial: Recent Advances in Bladder Cancer Diagnosis and Treatment. Front. Surg. 9:890172. doi: 10.3389/fsurg.2022.890172

Just to highlight some key messages from the articles. Chai et al. compared CxBladder, a new mRNA biomarker, with conventional urine cytology and found that the former had a high negative predictive value and sensitivity that accurately predicted suspicious cystoscopy findings. Sugeeta et al. reviewed current biomarkers and summarized the findings of each marker in clinical practice. Mulawkar et al. reviewed the role of the macroscopic image enhancement in the diagnosis of non-muscle-invasive bladder cancer which demonstrated a great utility in improving detection and short-term cancer control but no utility in delaying progression, or in long-term cancer control. Fankhauser et al. reviewed the current role of en bloc resection of bladder tumors, showing the improvement in clinical outcomes of the en bloc procedures. This result was confirmed by Liu et al. who showed that Thulium laser en bloc resection was safer than conventional transurethral resection with fewer perioperative complications. Asimakopoulos et al. analyzed the importance of T1 sub staging in non-muscle-invasive bladder cancer and showed that extensive invasion of the lamina propria was significantly associated with recurrence-free survival and progression-free survival. Jena et al. reviewed and analyzed the different anatomic templates of pelvic lymph node dissection during radical cystectomy, based on levels of pelvic lymph nodes. Mazzucchelli et al. provided important information on handling and reporting of the bladder cancer samples to improve the close collaboration between pathologists and urologists. Finally, Zhao et al. showed in a systematic review that intravesical chemohyperthermia had equivalent oncological outcomes and a similar safety profile when compared to BCG maintenance therapy for patients with intermediate- and high-risk non-muscle-invasive bladder cancer.

This Research Topic represents the hard work of all the authors and reviewers, who have contributed significantly to make this possible. We hope that you will enjoy reading this special Research Topic as we did in handling all the papers.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

ACKNOWLEDGMENTS

We would like to thank all researchers who have contributed to this Topic Issue and all reviewers who made this possible.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Surgery

ORIGINAL RESEARCH
published: 13 May 2021
doi: 10.3389/fsurg.2021.659292

Comparing CxBladder to Urine Cytology as Adjunct to Cystoscopy in Surveillance of Non-muscle Invasive Bladder Cancer—A Pilot Study

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *27 January 2021*
Accepted: *07 April 2021*
Published: *13 May 2021*

Citation:
Chai CA, Yeoh WS, Rajandram R, Aung KP, Ong TA, Kuppusamy S, Nazran A, Kumaran K, Razack AHA and Teoh JY (2021) Comparing CxBladder to Urine Cytology as Adjunct to Cystoscopy in Surveillance of Non-muscle Invasive Bladder Cancer—A Pilot Study. Front. Surg. 8:659292. doi: 10.3389/fsurg.2021.659292

Purpose: Guidelines advocate cystoscopy surveillance (CS) for non-muscle invasive bladder cancer (NMIBC) post-resection. However, cystoscopy is operator dependent and may miss upper tract lesions or carcinoma *in-situ* (CIS). Urine cytology is a common adjunct but lacks sensitivity and specificity in detecting recurrence. A new mRNA biomarker (CxBladder) was compared with urine cytology as an adjunct to cystoscopy in detecting a positive cystoscopy findings during surveillance cystoscopy in our center.

Materials and Methods: Consented patients older than 18, undergoing CS for NMIBC, provide paired urine samples for cytology and CxBladder test. Patients with positive cystoscopy findings would undergo re-Trans Urethral Resection of Bladder Tumor (TURBT).

Results: Thirty-five patients were enrolled from April to June 2019. Seven contaminated urine samples were excluded. The remaining cohort of 23 (82%) and 5 (18%) females had a mean age of 66.69 (36–89). Eight (29%) patients with positive cystoscopy finding underwent TURBT. All 8 patients also had positive CxBladder result. This shows that CxBladder has a sensitivity and negative predictive value (NPV) of 100%, specificity of 75% and positive predictive value (PPV) of 62% in predicting a positive cystoscopy finding. TURBT Histo-pathological findings showed Low-grade Ta NMIBC in one patient (4%), and 7 (25%) patients had inflammatory changes. Urine cytology was only positive in one patient with a positive cystoscopy finding. This led to a sensitivity of merely 13% and NPV of 74%, while specificity and PPV was 100% in predicting a positive cystoscopy finding.

Conclusion: CxBladder had high NPV and sensitivity which accurately predicted suspicious cystoscopy findings leading to further investigation. It has great potential for use as adjunct to cystoscopy for surveillance of NMIBC.

Keywords: bladder cancer, CxBladder, cystoscopy, non-muscle invasive bladder cancer, urine cytology

INTRODUCTION

Bladder cancer is identified as the 11th most commonly diagnosed cancer in the world (1, 2); of which 75% of patients presented initially as non-muscle invasive bladder cancer (NMIBC) (3). The EORTC Genito-Urinary Cancer group reported that non-muscle invasive bladder cancer is associated with a high recurrence rate after transurethral resection of bladder cancer (TURBT) of up to 80% in 5 years (4). Hence, various international guidelines such as the European Association of Urology (EAU), National Comprehensive Cancer Network (NCCN) and American Urological Association (AUA) guidelines advocate close monitoring and routine surveillance with cystoscopy as the current standard of care (5–7).

However, cystoscopy is operator dependent and upper tract lesions, early tumor or carcinoma *in-situ* (CIS) may sometimes be inconspicuous. Some literature recommend employing cystoscopy advancements such as fluorescence cystoscopy or narrow-band imaging in an attempt to assist in the identification of bladder cancer recurrence (8, 9). Currently, the commonly used adjunct to cystoscopy in surveillance of bladder cancer is urine cytology, even though studies have shown it lacks sensitivity and specificity in detecting bladder cancer recurrence. Furthermore, urine cytology is usually more accurate in detecting high-grade urothelial carcinoma, as evidence has shown a very low sensitivity for low-grade tumors (10–12).

A novel commercially available urine-based test (CxBladder) using mRNA biomarkers to detect bladder cancer recurrence in urine samples has been developed. CxBladder urine biomarkers have strong differential expression between tumors and normal bladder tissue, and the ability to identify broad inter-tumor heterogeneity known to bladder cancer (13). Although CxBladder was reported to hold better potential than urine cytology as an adjunct to cystoscopy, there is still limited studies to clearly establish CxBladder's superiority (14); hence it is still not a standard test in clinical guidelines especially in this region.

Therefore, this pilot study aims to compare CxBladder with urine cytology as an adjunct to cystoscopy in predicting positive cystoscopy findings leading to subsequent TURBT in surveillance of NMIBC.

MATERIALS AND METHODS

Design

This was a prospective, single-center cohort study. We recruited 35 patients from the University Malaya Medical Center, Kuala Lumpur, who underwent surveillance cystoscopies to rule-out recurrence of bladder cancer. The primary outcome is to predict a positive cystoscopy findings requiring subsequent TURBT using both CxBladder or Urine Cytology.

Ethical Approval

Ethics approval was acquired from University Malaya Medical Center ethics board (No.: 2019129-7072). This study was carried out following the principles of the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines.

Patients

Patients aged 18 years old or older undergoing surveillance cystoscopies after being diagnosed with primary or recurrent NMIBC during the past 2 years were eligible for this study. Upon consent, patients were given a 50 mls specimen container to provide a fresh voided midstream urine sample before undergoing cystoscopy. The collected urine was separated into paired urine samples and sent for urine cytology and the CxBladder test. Patients who had active urinary tract infections were excluded from this study.

Assessment

The CxBladder assesses the probability of bladder cancer recurrence by extracting and quantifying 5 mRNA biomarkers which are believed to be in higher concentrations in urine sample of bladder cancer patients (13). The biomarkers genes identified are: MDK, HOXA13, CDC2, IGFBP5, CXCR2. This is achieved by reverse transcription (RT) quantification polymerase chain reaction (13).

Results acquired from this study were entered into prespecified data collection forms before analysis. The results from CxBladder test kits were mailed to the investigator in a registered sealed document courier service as well as in a password encrypted email to ensure patients' data confidentiality.

Patients with positive cystoscopy findings were counseled and subjected to re-TURBT and treatment as per clinical guidelines. Patients with negative cystoscopy findings but positive urine cytology and/or CxBladder would undergo a repeat cystoscopy examination at an earlier date with a CT scan to rule out extravesical recurrence.

Statistical Analysis

The comparative performance of CxBladder and cytology was analyzed by calculating the negative predictive value (NPV) and positive predictive value (PPV) of each. Sensitivity and specificity were also calculated for comparative purposes. These performance metrics, Numerical were represented as median $\pm$ standard deviation and/or frequencies using Microsoft Excel. These performance metrics and their 95% confidence intervals (CI) were calculated by standard methods using an online calculator.[1]

RESULTS

Patients Characteristic

A total of 35 patients who were under follow up for bladder cancer surveillance consented for this study between April to June 2019. Seven were excluded from this study after urine samples sent were contaminated and deemed not suitable for CxBladder analysis as they were suggestive of urinary track infections or proteinuria (**Figure 1**).

Out of 28 remaining bladder cancer patients, 82% ($n = 23$) were males and 18% ($n = 5$) were females. The median age was 70-years-old (range: 59–74) (**Table 1**). The majority of the

[1]https://www2.ccrb.cuhk.edu.hk/stat/confidence%20interval/Diagnostic%20Statistic.htm.

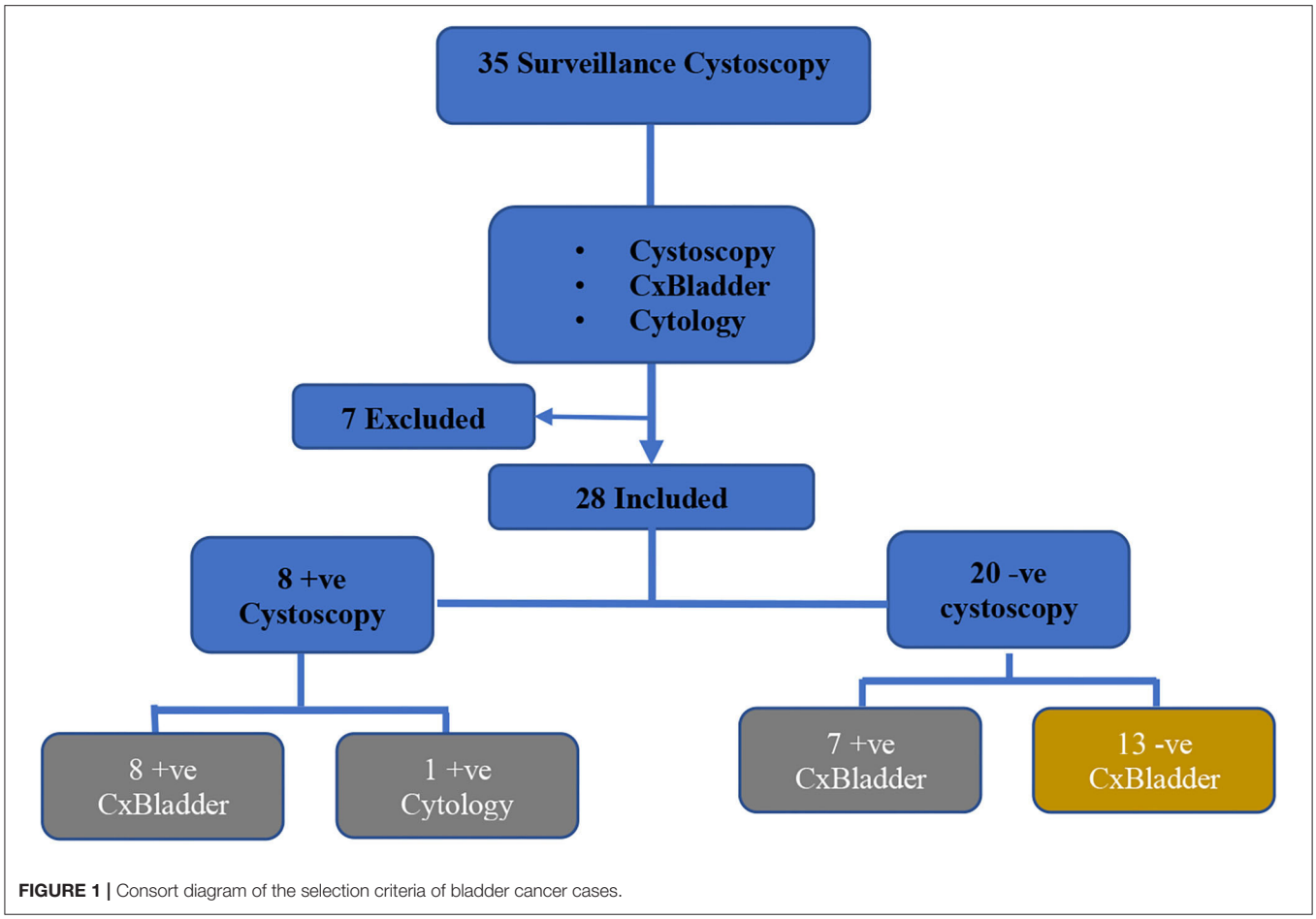

FIGURE 1 | Consort diagram of the selection criteria of bladder cancer cases.

patients with non-muscle invasive bladder cancer consisted of Chinese ethnicity (61%, $n = 17$), followed by Indian (21%, $n = 6$), Malay (15%, $n = 4$) and another race (3%, $n = 1$). Sixty one percentage ($n = 17$) of these patients were smokers.

Tumor Characteristic

Examination of the tumor characteristic (T-staging) showed almost equal distribution of Ta ($n = 15$) and T1 ($n = 13$). The grade of the lesion in our cohort showed predominantly high grade ($n = 18$) tumor compared to low grade ($n = 10$) disease. Distribution according to risk group shows 32% low risk disease ($n = 9$), 25% intermediate risk disease ($n = 7$) and 43% ($n = 12$) high risk disease (**Table 1**). Four patients had history of CIS in previous histopathological findings. Fifty four percentage ($n = 15$) of these patients received intravesical BCG while 35% ($n = 10$) received intravesical mitomycin during the course of treatment.

Cystoscopy Finding

Twenty nine percentage ($n = 8$) of the bladder cancer patients had cystoscopy findings suspicious of recurrence and underwent TURBT. Of these, all patients had positive CxBladder findings. The histopathological findings showed 3% ($n = 1$) low-grade Ta recurrent bladder cancer, and 25% ($n = 7$) inflammatory changes. Three percent ($n = 1$) of patient in whom CxBladder test was positive, displayed positive urine cytology as well, but with a negative histopathological outcome (**Figure 1**). Among 71% ($n = 20$) patients with negative cystoscopy findings; 25% ($n = 7$) of them had a positive CxBladder result (**Figure 1**). These cases were scheduled for early repeat cystoscopy for reassessment before CT imaging to rule out extravesical recurrence.

CxBladder showed a very high sensitivity (100%) and negative predictive value (NPV) (100%) [95% CI = 2.86 (2.85714–2.85715)] in detecting a positive cystoscopy finding as opposed to urine cytology which showed a mere sensitivity of 13% and a negative predictive value of 74% (**Table 2**).

Conversely, urine cytology demonstrated a much higher specificity and PPV (100%) while CxBladder demonstrated much lower specificity (75%) and PPV (62%) in predicting a positive cystoscopy finding.

Relations Between Previous Intravesical Mitomycin C/BCG and CxBladder

In our cohort, 35% ($n = 10$) of the patients received intravesical mitomycin for previous low-grade histology of bladder cancer. Of these, four patients (14%) showed positive CxBladder test while six (21%) were negative.

TABLE 1 | Patient demographics of CxBladder surveillance.

	No (%)	Positive CxBladder	Negative CxBladder
Median age + IQR	70 (59–74)		
Gender			
Male	23 (82%)	12 (43%)	11 (39%)
Female	5 (18%)	3 (11%)	2 (7%)
Race			
Chinese	17 (61%)	9 (32%)	8 (29%)
Malay	4 (15%)	3 (12%)	1 (3%)
Indian	6 (21%)	2 (7%)	4 (14%)
Others	1 (3%)	1 (3%)	
Risk			
Smoker	17 (61%)	9 (32%)	8 (29%)
Bladder Ca Staging			
Ta	15 (54%)	8 (29%)	7 (25%)
T1	13 (46%)	7 (25%)	6 (21%)
Bladder Ca grading			
High grade	18 (64%)	8 (29%)	10 (35%)
Low grade	10 (36%)	7 (25%)	3 (11%)
Bladder Ca risk group			
Low risk	9 (32%)	4 (14%)	5 (18%)
Intermediate risk	7 (25%)	4 (14%)	3 (11%)
High risk	12 (43%)	7 (25%)	5 (18%)
Previous intravesical therapy			
Mitomycin	10 (35%)	4 (14%)	6 (21%)
BCG	15 (54%)	8 (29%)	7 (25%)
N/A	3 (11%)	3 (11%)	

TABLE 2 | CxBladder vs. urine cytology for bladder cancer cases.

	CxBladder	Cytology
Sensitivity	100%	13%
Specificity	75%	100%
Positive Predictive Value	62%	100%
Negative Predictive Value	100%	74%
95% C.I	2.86 (2.85714–2.85715)	

Similarly, for patients with high grade histology of bladder cancer who received prior intravesical BCG therapy; the result of CxBladder were fairly equally distributed with eight patients (29%) having positive CxBladder and seven (25%) displayed negative result.

DISCUSSION

This is a pilot study in our local center to access the feasibility of using CxBladder compared to urine cytology as an adjunct to white light cystoscopy for post-operative surveillance of non-muscle invasive bladder cancer. We compared the likelihood of each to predict a positive cystoscopy finding requiring TURBT. A histologically proven malignancy is the only way to confirm the diagnosis of recurrent bladder cancer, this can only be done by performing a TURBT in the event of a postitive cystoscopy finding (15). Hence, the ability to first predict a positive cystoscopy finding will have the potential to serve as a screening tool to "rule-out" the need for invasive cystoscopy examination.

Our study had shown that CxBladder had a significantly higher NPV (100%) [95% CI = 2.86(2.85714–2.85715)] in detecting bladder cancer recurrence. This coincides with previously reported studies which identified CxBladder as a superior "rule-out" test than urine cytology (14). In a study by Kavalieris et al. among 1036 patients, it was reported that CxBladder has a sensitivity of 93% and NPV of 97% in detecting bladder cancer recurrence. Therefore, in patients who are unable to tolerate a cystoscopy or demonstrated suspect compliance to regular cystoscopy surveillance, CxBladder may play a role as an initial surveillance tool to "rule out" before submitting the patient to cystoscopy which is currently the standard of care (5–7). This can also be especially useful in rare cases of patients with underlying spinal cord injury where flexible cystoscopy might lead to complications such as autonomic dysreflexia (16).

The potential use of CxBladder as a screening tool with high NPV could gain importance especially in the Covid-19 pandemic era. A non-invasive screening with CxBladder could reduce the unnecessary exposure of patients to health-care workers and environment which could subsequently reduce the risk of Covid-19 infection. Furthermore, reduction of cystoscopy procedures plus full personal protective equipment (PPE) use can potentially be more cost effective in the future.

Recently, a prospective randomized study has found that prior knowledge of positive urine cytology may improve the quality of follow-up cystoscopy (17). Madelon et al. described in their study that diagnostic review bias may increase scrutiny of cystoscopic examination during cystoscopy surveillance for NMIBC. Furthermore, Madelon et al. did not notice an increase in false positive cystoscopy findings in patients who had a positive urine test (17). Hence, supporting the adjunctive role of CxBladder performed before follow up surveillance cystoscopy.

In our cohort of patients, there were 25% ($n = 7$) false-positive results for CxBladder. These patients showed negative cystoscopy findings despite a positive CxBladder result.

We postulated that CxBladder may be very sensitive to inflammatory changes in the bladder. However, we cannot confirm whether this is indeed a false positive CxBladder result, or due to a high pickup of microsatellite recurrence where white light cystoscopy failed to pick up disease recurrence. Even though some guidelines advocate random biopsy for patients with discordance of positive urine cytology and negative cystoscopy findings, urine cytology results for these seven patients were also negative. Hence, we decided to avoid possible invasive complications and over treatment by arranging earlier cystoscopy appointment to ensure no tumor recurrence is missed.

The main limitation of our study is the small number of patients in our cohort. However, our results coincide with those of larger studies in demonstrating the high sensitivity and NPV of CxBladder in predicting a positive cystoscopy findings (14, 16). As this was a pilot study, the small number of bladder cancer patients was sufficient to prove that CxBladder is a feasible adjunct to cystoscopy in our local population. A larger cohort will be needed to further support our findings. Long-term follow-up as per standard guidelines for NMIBC will provide more robust

data on the feasibility of employing CxBladder as the preferred adjunct compared to urine cytology in the near future.

CONCLUSION

Cxbladder has high NPV and sensitivity in predicting the chances of a suspicious cystoscopy finding that will lead to further investigation. In-depth study using CxBladder on a larger cohort will be needed to justify these findings as it may offer less invasive surveillance for bladder cancer before proceeding with cystoscopy examination. Further investigation of patients who have negative cystoscopy findings with a positive CxBladder result will provide more data in assessing the sensitivity of CxBladder in picking up occult bladder cancer recurrence.

DATA AVAILABILITY STATEMENT

The original contributions presented for this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University Malaya Medical Center ethics board (No.: 2019129-7072). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

CAC wrote the manuscript, designed, and carried out this study. WSY, AN, and KPA performed the cystoscopy examinations, read, and approved the menuscript for submission. RR performed the statistical analysis, read, and approved the menuscript for submission. TA, SK, KK, and AR, read, edited, and approved the manuscript for submission. JT reviewed, edited, and approved the manuscript for submission. All authors contributed to the article and approved the submitted version.

FUNDING

The authors declare that this study received funding from Pacific Edge Limited. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Surgery

MINI REVIEW
published: 31 May 2021
doi: 10.3389/fsurg.2021.685506

En Bloc Resection of Bladder Tumor—Is It the Way Forward?

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Transurethral resection of bladder tumors (TURBT) represents the cornerstone in diagnosis and treatment of bladder cancer but recurrence is observed in up to 80% and over- or understaging with TURBT is common. A more recent development to overcome these limitations represents en-bloc resection of bladder tumors (ERBT) which offers several advantages over TURBT. In this report, we briefly review studies assessing outcomes of bladder cancer patients undergoing ERBT. Most randomized and non-randomized trial demonstrate improvement in clinical outcomes for ERBT over TURBT, however more pathological and translational studies are warranted.

Keywords: trans-urethral resection of bladder tumor, bladder cancer, transurethral, urothelial atypia, urothelial cancer

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *25 March 2021*
Accepted: *30 April 2021*
Published: *31 May 2021*

Citation:
Fankhauser CD, Wettstein MS, Afferi L, Grossmann NC and Mostafid H (2021) En Bloc Resection of Bladder Tumor—Is It the Way Forward? Front. Surg. 8:685506. doi: 10.3389/fsurg.2021.685506

INTRODUCTION

Bladder cancer has an estimated 429,793 new diagnoses leading to 165,084 deaths per year worldwide (1) but with a globally wide variation (2). For example in the European Union, an increase by 41% from currently 124 188 up to 174 891 new cases per year is expected by the year 2035 (1). Patients with non-muscle invasive bladder cancer (NMIBC) require lifetime surveillance with cystoscopy and imaging. Due to the high recurrence-rate of up to 80% and related re-treatment, lifetime treatment costs are among the highest of all cancers, ranging from $100,000 to $200,000 per patient in the United States (3). This significant financial burden on the population and healthcare system calls for effective bladder cancer diagnostics and treatment.

Transurethral resection of bladder tumors (TURBT) represents the cornerstone in diagnosis and treatment of bladder cancer since the 1940s. Aims of TURBT include symptom relief, histological diagnosis (grade, stage, variant histology) and cure in some stages of NMIBC. Several technological advantages including bipolar TURBT have been introduced (4). However, both mono- and bipolar TURBT represent a piecemeal resection of the tumor with the risk of tumor seeding and over- and under staging because of tangential sectioning and thermal artifacts (5). This resection method ignores basic principle of oncological surgery being resection in one specimen instead of scattering malignant cells. To decrease the high recurrence rates, several new methodologies have been developed to allow en bloc removal of bladder cancers, for example in 1980 snare polypectomy a method which did not allow complete resection of the bladder base (6).

Complete en-bloc resection including the bladder base was only achieved after the introduction of en-bloc resection techniques as described in 1997 by Kawada et al. using a new arched resection loop (7). Later, the same approach but using a standard mono- or bipolar loop electrode or laser fibers have been introduced. A recent consensus agreed that any method "removing the bladder tumor in one piece" can be described as en-bloc transurethral resection of bladder tumors (ERBT) (8). All ERBT techniques have three assumed advantages. First, the histological assessment may be facilitated by en-bloc resection. Second, remaining in the same surgical plain may decrease

complications. Third, avoiding tumor fragmentation may decrease the tumor spillage and improve oncological outcomes. In this report, we review studies assessing outcomes of patients with bladder cancer undergoing ERBT.

In the most recent and comprehensive systematic review and meta-analysis, Teoh et al. identified 10 randomized controlled trials comparing ERBT with TURBT (8). Limitations of those trials included low individual sample size as well as heterogeneity in outcome reporting and treatment (e.g., energy source used, postoperative management). Nevertheless, this meta-analysis represents the best available evidence and the authors concluded that compared to TURBT, ERBT has a longer operation time but shorter irrigation time and lower risk of bladder perforation. However, this meta-analysis of randomized trials was not able to show a difference regarding catheterization time, hospital stay, occurrence of obturator nerve reflex, presence of detrusor muscle in specimen or recurrence rates. Two more recent randomized trials were not included in the review. The first compared classical TURBT with ERBT using hydrodissection, both assisted by photodynamic diagnosis using hexaminolevulinate (HAL), and reported a higher percentage of presence of detrusor muscle in specimen in the en-bloc group (86 vs. 63%) (9).The second compared TURBT with holmium laser ERBT and reported a higher rate of post-operative epirubicin instillations, shorter time to catheter removal and hospital stay with a higher percentage of presence of detrusor muscle, fewer cautery artifacts and residual tumor in the pathological specimen but no difference in recurrence free survival (10).

In the same systematic review, the authors also compared the results of 22 non-randomized trials with similar findings regarding irrigation time and bladder perforation rates but discordant and more favorable results for ERBT regarding catheterization time, hospital stay, occurrence of obturator nerve reflex, presence of detrusor muscle in specimen and recurrence rates. Those findings are in line with similar studies which were published more recently and were therefore not included in the systematic review (11–14). Additionally, the authors performed a Delphi consensus and reached consensus that ERBT can be attempted in patients with <4 bladder tumors with a tumor size <3 cm. Consensus was also reached in other key areas including the statement that marking of the planned circumferential margin at least 5 mm from any visible bladder tumor before starting the resection is recommended. In order to assess the feasibility of ERBT in routine practice the same authors implemented ERBT as the primary surgical approach in all NMIBC patients with TURBT reserved as a conversion procedure in those patients where ERBT could not be completed for technical reasons. The authors found that ERBT was successfully carried out in 73% of all patients including those with large and multi-focal tumors, and 84% in patients with bladder tumors of ≤ 3 cm confirming that ERBT could be used as the primary approach for excising bladder tumor in the majority of NMIBC patients (15).

Whereas, direct improvements of certain clinical outcomes by ERBT are suggested by numerous clinical studies, pathological and translational studies are limited. First pathology studies suggested a higher interobserver concordance and time for analysis for ERBT specimens compared to TURBT (16) and the potential for improved sub staging in T1 disease (17). A second translational study reported a higher level of circulating tumor cells after TURBT compared to ERBT (18). Whilst the technique of ERBT has been relatively standardized irrespective of energy source used, the major current limitation of ERBT remains extraction of large en-bloc specimens, generally >3 cm. A number of techniques have been described such as the use of an Endo-catch specimen retrieval bag. Such challenges could be overcome fairly easily with collaboration from endoscopic equipment manufacturers (19).

In summary, en-bloc TURBT seem to be comparable or superior in most outcomes compared to classical TURBT and those results seem to be compelling for many urologists. A recent survey among 200 European urologists which reported that en-bloc TURBT is already the resection technique of choice in 35% of cases (20). Further development and studies of this technique are warranted.

AUTHOR CONTRIBUTIONS

CF and HM: manuscript drafting and literature review. LA, MW, and NG: critical revision of manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Surgery

REVIEW
published: 17 June 2021
doi: 10.3389/fsurg.2021.687636

The Adequacy of Pelvic Lymphadenectomy During Radical Cystectomy for Carcinoma Urinary Bladder: A Narrative Review of Literature

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An adequate pelvic lymph node dissection (PLND) is an essential part of radical cystectomy for muscle invasive bladder cancer. However, the definition of what constitutes an adequate PLND is often shrouded in controversy. Various authors have defined different anatomic templates of PLND based on levels of pelvic lymph nodes. Some have suggested other surrogate markers of the adequacy of PLND, namely lymph node count and lymph node density. While individual studies have shown the efficacy and reliability of some of the above markers, none of them have been recommended forthright due to the absence of robust prospective data. The use of non-standardized nomenclature while referring to the above variables has made this matter more complex. Most of older data seems to favor use of extended template of PLND over the standard template. On the other hand, one recent randomized controlled trial (RCT) did not show any benefit of one template over the other in terms of survival benefit, but the study design allowed for a large margin of bias. Therefore, we conducted a systematic search of literature using EMBASE, Medline, and PubMed using PRISMA-P checklist for articles in English Language published over last 20 years. Out of 132 relevant articles, 47 articles were included in the final review. We have reviewed existing literature and guidelines and have attempted to provide a few suggestions toward a uniform nomenclature for the various anatomical descriptions and the extent of PLND done while doing a radical cystectomy. The results of another large RCT (SWOG S1011) are awaited and until we have a definitive evidence, we should adhere to these suggestions as much as possible and deal with each patient on a case to case basis.

Keywords: bladder cancer, pelvic lymphadenectomy, extended lymphadenectomy, pelvic lymph node dissection, super extended pelvic lymphadenectomy

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *29 March 2021*
Accepted: *19 May 2021*
Published: *17 June 2021*

Citation:
Jena R, Shrivastava N, Sharma AP, Choudhary GR and Srivastava A (2021) The Adequacy of Pelvic Lymphadenectomy During Radical Cystectomy for Carcinoma Urinary Bladder: A Narrative Review of Literature. Front. Surg. 8:687636. doi: 10.3389/fsurg.2021.687636

INTRODUCTION

Each year, more than 400,000 patients worldwide are diagnosed with bladder cancer of which ∼30% are muscle invasive (1). Radical cystectomy (RC) with bilateral pelvic lymph node dissection (PLND) is the standard of care for recurrent high risk non muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC) (2, 3). Preoperative cross-sectional imaging, with a sensitivity of about 52% for positive pelvic lymph nodes, often leads to significant under staging (4–6). A thorough bilateral PLND therefore increases the staging procedure's accuracy and provides a probable survival benefit in patients of MIBC irrespective of the nodal involvement (7, 8). Now the question arises as to how should one assess the adequacy of PLND? Whether it is the levels of pelvic lymph nodes removed, the anatomical template of dissection followed, the lymph node count or the lymph node density remains a bone of contention? Adding to this confusion, is the frequent use of non-standardized nomenclature in denoting the extents of PLND across various studies. Therefore, we attempted to review the existing literature related to the levels of pelvic lymph nodes and the various templates of PLND defined by different authors to bring clarity to this issue. We also suggest certain points which can bring a uniformity to this procedure and thus facilitate better reporting of the outcomes of PLND for MIBC.

METHODOLOGY

Search Strategy and Inclusion Criteria

The systematic literature search was done for relevant papers, by two authors RJ and APS, in various electronic databases as follows. The following keywords with the operators from the years 2001 to 2020 were used: ("lymph node*" OR standard OR extended OR lymphadenectomy) AND (("bladder cancer" OR "bladder carcinoma" OR urothelial) AND cancer OR "urothelial carcinoma of the bladder") AND radical AND cystectomy for searching EMBASE/Medline and the following keywords were used for searching PubMed - (lymphadenectomy) OR (Standard) OR (extended) OR (lymph node) AND ((bladder cancer) OR (urothelial cancer) OR (bladder carcinoma) OR (urothelial carcinoma)) AND (radical cystectomy). The conference abstracts, conference papers, conference review, erratum, and notes were removed and the search results were filtered to include the articles only in English Language.

Results

Initial search yielded 4,604 articles from EMBASE and 202 results from PUBMED. After applying exclusion criteria, this narrowed the number of articles to 98 from Embase. After removing the duplicates a total of 132 articles (EMBASE+PUBMED) were assessed by two authors (APS and RJ) independently. The reference list was searched for more relevant articles. Total of 47 articles were selected for review of literature (**Table 1**). The selection process is outlined in **Figure 1**. The current narrative review is based on these 47 included articles.

Anatomy of Lymphatic Drainage of Bladder

The primary drainage site for the bladder consists of the external iliac, internal iliac, obturator, and presacral lymph nodes. The secondary drainage goes to the common iliac, para-aortic, inter-aortocaval, and para-caval lymph nodes (**Figure 2**) (9). Bilaterality of lymph nodal spread has been demonstrated in up to 39% of patients. It has been confirmed using single- photon emission computed tomography (SPECT) and an intraoperative γ-probe after injection with a technetium nano-colloid, which has also shown that up to 52% of nodes may lie outside the true pelvis (10–12). Leissner et al. (13) have identified 12 anatomical sites with variable probability of metastatic deposits, with the obturator groups being the commonest involved site. In this study, 6.9% patients had metastases in the regions above the common iliac bifurcation and 2.9% had metastases in the inter-aortocaval and precaval regions. Many subsequent studies have shown that up to 41% of positive lymph nodes lie above the bifurcation of the common iliac arteries (14). In 591 patients, Tarin et al. (15) reported lymph node involvement in 1194 patients (19%). Of these, seven patients (6%) had no positive lymph nodes within the true pelvis (skip lesions). Since skip lesions are known to be very rare, this phenomenon may be the result of missed positive lymph nodes in the true pelvis or of a specimen-labeling error. But a few things are clear. First, PLND should be bilateral since drainage is bilateral. Next, a limited PLND template has a small but significant chance of missing positive nodes lying outside the true pelvis. However, whether wider dissection necessarily translates into oncological advantage needs to be seen.

Anatomical Variables Used to Assess Adequacy of Pelvic Lymph Node Dissection

Levels of Pelvic Lymph Node Dissection

In their paper in 2004, Leissner et al. (13) proposed a 3-tier classification system for the extent of PLND during radical cystectomy (**Figure 3**). The anatomical sites and their boundaries have been described in **Table 2**. In the more contemporary studies, this system of denoting extent of PLND has been used sparingly compared to the anatomical templates discussed in the next section (16).

Templates of Pelvic Lymph Node Dissection

The EAU Working Group on MIBC proposed the following nomenclature for the anatomical templates used in PLND based on the recommendations of an expert panel: *limited*, *standard*, *extended*, and *super-extended PLND*. The definition of these terms has been rather inconsistent and studies have often termed anything less than an extended template as a limited template. Limited PLND typically includes dissection restricted to the bilateral obturator fossae (**Figure 4A**) (14). Boundaries of standard PLND include the common iliac bifurcation cranially and the inguinal ligament caudally. Laterally the boundaries are the genitofemoral nerve and medially it is the bladder wall. This template typically includes the distal common iliac, the external iliac, the obturator and the internal iliac lymph nodes bilaterally (**Figure 4B**) (14, 17). In addition to all the lymph

TABLE 1 | Details of articles selected for review of literature.

No.	References	Year	Conclusion
1	Funt and Rosenberg (1)	2017	The standard of care for muscle invasive bladder cancer is neoadjuvant cisplatin based chemotherapy followed by radical cystectomy and bilateral pelvic lymphadenectomy.
2	Buscarini et al. (2)	2007	Extended pelvic lymph node dissection during radical cystectomy provides diagnostic and therapeutic benefit on muscle invasive carcinoma bladder.
3	Sung and Lerner (3)	2020	The first randomized phase III trial did not show benefit of extended pelvic lymphadenectomy. However, there are many potential shortcomings of this trial. The results of the SWOG 1011 trial should be able to give us a better idea about the benefits of an extended template of dissection.
4	Papalia et al. (4)	2012	Diffusion weighted MRI can differentiate between metastatic and non-metastatic pelvic lymph nodes in patients with high grade bladder cancer.
5	Crozier et al. (5)	2019	PET-CT and MRI are more sensitive than CT scan for detection of positive lymph nodes in bladder cancer prior to cystectomy.
6	Jeong et al. (6)	2015	Combined PET-CT does not have increased sensitivity compared to CT alone for the detection of positive pelvic lymph nodes in patients of bladder cancer prior to radical cystectomy.
7	Bruins et al. (7)	2014	Any pelvic lymph node dissection is better than no pelvic lymph node dissection. Extended dissection seems to be more advantageous than standard dissection. However super extended dissection doesn't provide additional therapeutic or diagnostic benefits.
8	Suttman et al. (8)	2007	Retrospective studies point out that while the benefit of a bilateral pelvic lymphadenectomy during radical cystectomy is unquestionable,
9	Cattaneo et al. (9)	2018	Extended pelvic lymph node dissection provides optimal diagnostic and therapeutic benefit in patients undergoing radical cystectomy for muscle invasive bladder cancer.
10	Abol-Enein et al. (10)	2004	The internal iliac and obturator group of lymph nodes are the sentinel group for bladder cancer. Bilateral dissection of these areas is mandatory. Negative nodes here mean that more proximal dissection is not necessary.
11	Bochner et al. (11)	2004	Extended template pelvic lymph node dissection had a significantly higher lymph node lymph node yield compared to standard dissection even though it doesn't provide any staging advantage.
12	Roth et al. (12)	2010	Standard template of pelvic lymph node dissection removes only 50% of all lymph nodes in the primary landing sites of bladder cancer while extended lymphadenectomy removes about 90%.
13	Leissner et al. (13)	2004	Extended radical cystectomy should be the standard of care in all patients of radical cystectomy. No sentinel lymph nodal area was identified.
14	Perera et al. (14)	2018	Extended pelvic lymphadenectomy provides optimal recurrence free and cancer specific survival. Super extended template provides no actual benefit. Increased lymph node yields provides improved oncological outcomes in patients with both node positive or node negative disease.
15	Tarin et al. (15)	2012	Pathological involvement of the common iliac lymph node is not associated with a worse outcome compared to the primary nodal basin disease, thus promoting the inclusion of this group in the primary pathological staging of bladder cancer during radical cystectomy. However number of positive lymph nodes was an independent predictor of poor outcomes.
16	Hwang et al. (16)	2019	Extended pelvic lymphadenectomy may reduce the risk of death from any cause in patients undergoing radical cystectomy for bladder cancer over time compared to standard pelvic lymphadenectomy. However there is a possibility of no effect.
17	Sundi et al. (17)	2014	Extended pelvic lymphadenectomy seems to be adequate for staging and cancer related outcomes. However, the super extended template may be associated with greater morbidity. Risk based approach should be followed to determine template of dissection in each patient.
18	Dorin et al. (18)	2011	Extended pelvic lymphadenectomy with meticulous dissection is more important that total lymph nodal count to achieve optimal oncological outcomes because lymph node metastases outside the boundaries of the standard template are common.
19	Dhar et al. (19)	2008	Extended pelvic lymph node dissection during radical cystectomy allows for more accurate staging and improved survival in patients with node positive and non-organ confined disease.
20	Li et al. (20)	2016	Greater number of dissected lymph nodes are associated with better survival advantages in patients of bladder cancer. Number of dissected lymph nodes could be an independent prognostic factor.
21	Bi et al. (21)	2014	Extended pelvic lymphadenectomy provides better recurrence free survival compared to standard lymphadenectomy in patients with both pathologically positive and negative pelvic lymph nodes.
22	Mandel et al. (22)	2014	Extended pelvic lymphadenectomy has better oncological outcomes and is not associated with greater perioperative mortality or higher complication rates.
23	Wang et al. (23)	2019	Extended pelvic lymphadenectomy has better recurrence free survival and disease specific survival in bladder cancer and is not associated with more postoperative complications compared to non-extended lymphadenectomy.
24	Zehnder et al. (24)	2011	Meticulous extended lymphadenectomy with emphasis on skeletonization of the pelvic vessels has shown to be similar to super extended lymphadenectomy in terms of oncological outcomes. Certain groups with suspicious lymph nodes even after neoadjuvant therapy may need more extensive dissections.

(Continued)

TABLE 1 | Continued

No.	References	Year	Conclusion
25	Møller et al. (25)	2016	Super extended lymphadenectomy may benefit only a small subgroup of patients with non-organ confined disease without macrometastases and is not beneficial in the general set of patients.
26	Holmer et al. (26)	2009	Extended lymph node dissection seems to have improved time to recurrence and survival, especially in patients with non-organ confined disease.
27	Simone et al. (27)	2013	Extended pelvic lymphadenectomy has significant staging accuracy and survival benefit for bladder cancer across all stage groups.
28	Abdi et al. (28)	2016	Extended pelvic lymphadenectomy appeared to reduce the risk of local recurrence but had no effect on overall survival. It was associated with higher blood loss but similar rates of complications.
29	Hugen et al. (29)	2010	Lymphovascular invasion, perineural invasion and lymph node yield <14 are independent risk factors for bladder cancer recurrence in patients with node negative bladder cancer.
30	Muilwijk et al. (30)	2018	Super extended lymph node dissection has no advantage compared to standard template. However by using a super extended template, we identify 2% more patients as node positive, which would have been falsely diagnosed as node negative using the standard template and resect 35% more positive LNs, which would have been left behind by standard template lymphadenectomy, with a limited increase in morbidity.
31	Gschwend et al. (31)	2019	Lymphadenectomy up to the inferior mesenteric artery failed to show any significant advantage over the standard lymph node dissection in terms of recurrence free survival, cancer specific survival or overall survival.
32	Lerner et al. (32)	2019	Editorial commentary on the LEA trial (31). The study was underpowered to detected smaller benefits that can be attributed to super extended dissection compared to the standard template because of its sample size. Also, since the survival curves showed some divergence toward the end of the follow up period, longer follow up is necessary to get further insights. Also the study was not designed to prove that the limited lymphadenectomy is not inferior to the extended lymphadenectomy.
33	Josephson et al. (33)	2005	Extended template of pelvic lymph node dissection provides greater therapeutic and diagnostic benefit.
34	Boström et al. (34)	2020	Identified clinical markers of morbidity, mortality and survival in patients of bladder cancer treated with radical cystectomy, of which extra nodal extension conferred a poor prognosis.
35	Chou et al. (35)	2016	Extended dissection may confer survival and recurrence free advantages. Neoadjuvant cisplatin based chemotherapy appears to decrease mortality compared to radical cystectomy alone.
36	May et al. (36)	2011	Removal of higher number of lymph nodes is associated with improved oncological outcomes. Use of an extended template of dissection along with assessment of lymphovascular invasion is essential in stratifying patients into risk groups and to identify those who might benefit from adjuvant therapy.
37	Morgan et al. (37)	2012	Lymph node count at radical cystectomy is a predictor of overall survival and disease specific survival in patients with pathologically node negative disease but not in patients with pathologically positive lymph nodes.
38	Herr et al. (38)	2002	A greater number of lymph nodes is associated with a better staging and impact patient outcomes. Along with therapeutic and staging benefits it also helps identify patients who would benefit from adjuvant therapy.
39	VAN Bruwaene et al. (39)	2016	Predictors like total number of lymph nodes, number of positive lymph nodes, lymph node density and presence of extra nodal extension along with tumor characteristics like T stage and histology and neoadjuvant chemotherapy should be incorporated into normograms used for prognosticating patients who have undergone radical cystectomy.
40	Matsumoto et al. (40)	2015	Extended pelvic lymph node dissection helps in improving prognosis by eliminating micrometastases.
41	Cha et al. (41)	2015	There is no concrete evidence to favour extended pelvic lymphadenectomy over standard lymphadenectomy alone.
42	Capitanio et al. (42)	2009	Removing a minimum of 25 lymph nodes confers a 75% probability of detecting lymph node metastases and removing atleast 45 nodes gives a 90% probability. 15 lymph nodes have 50% probability and thus the goal is that atleast 25 lymph nodes should be removed during radical cystectomy.
43	Koppie et al. (43)	2006	There is no minimum lymph nodal count that can optimize outcomes after radical cystectomy. However increasing nodal yield is associated with increasing probability of survival. This highlights that extended lymphadenectomy should be done to improve outcomes.
44	Ku et al. (44)	2015	Lymph node density is an independent predictor of clinical outcome in lymph node positive patients after radical cystectomy.
45	Lee et al. (45)	2012	Lymph node density is an useful tool for risk stratifying patients after radical cystectomy and higher lymph node density has poorer disease specific survival in node positive patients.
46	Kondo et al. (46)	2012	Extended lymph node dissection improves oncological outcomes after radical cystectomy. Lymph node density is an important predictor of overall survival in node positive patients.
47	Ahn et al. (47)	2015	Extracapsular extension is an important prognostic factor for node positive bladder cancer.

nodes removed in the standard template, extended dissection entails removing the presacral nodes and all the nodes between the aortic and the common iliac bifurcations (**Figure 4C**) (14, 17). Super-extended PLND template involves removal of all the nodal tissue caudal to the base of the inferior mesenteric artery (**Figure 4D**) (14).

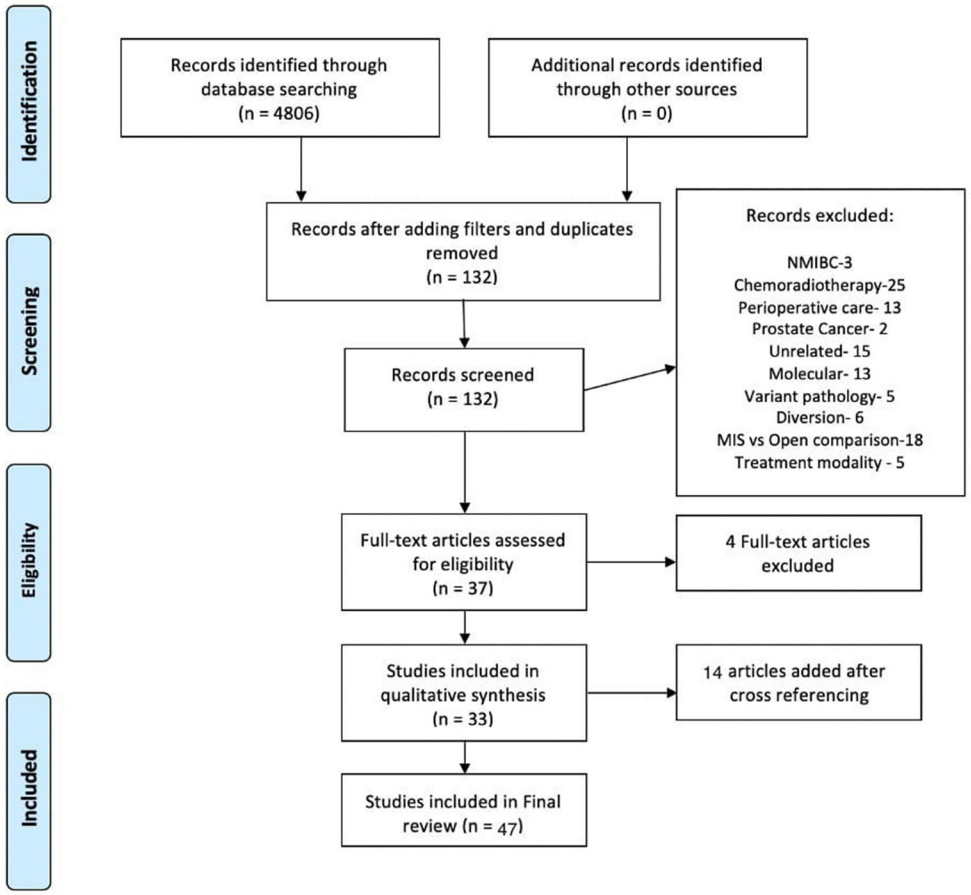

FIGURE 1 | Flowchart showing search strategy and selection of articles for review.

Adequate Pelvic Lymph Node Dissection

Limited vs. Extended PLND

Limited PLND has been shown to miss about 50% of positive lymph nodes in muscle invasive bladder cancer (12). The rationale behind performing a standard template PLND is that 90–95% of all node positive patients can be identified (13, 18). Dhar et al. (19) retrospectively compared limited PLND to extended PLND in 658 patients across 2 centers. It is important to point out here that what the authors describe as a limited PLND in their report is actually a standard template PLND we have described earlier. This discrepancy is an example of how a non-standardized system of nomenclature is fraught with confusion. In this study, 26% node positive patients were identified in the extended template cohort compared to 13% in the limited PLND cohort. Five-years recurrence free survival (RFS) was 23 vs. 57% ($p < 0.0001$), and overall survival (OS) was 26 vs. 46% ($p = 0.0021$), in favor of the extended LND group. For node positive patients the 5-year relapse-free survival and overall survival were both 7% for a limited dissection compared with 35 and 34% for patients undergoing extended LND, respectively ($p < 0.0001$). The authors concluded that standard PLND is associated with suboptimal staging and poorer outcomes for node positive and node negative disease with comparable pT stage and a higher rate of local progression, as summarized in **Table 2**.

In a meta-analysis by Li et al. (20), a greater extent of LND during RC had statistically significant advantages in OS, CSS and RFS, corresponding to reduced risks of 28, 34, and 36%, respectively. Bi et al. (21) showed that extended PLND was associated with improved RFS (HR = 0.66, 95% CI: 0.56–0.78, and $p < 0.001$) (21). The benefits of extended PLND were present in node-negative disease (HR = 0.68, 95% CI: 0.51–0.90, and $p = 0.007$), node-positive disease (HR = 0.58, 95% CI: 0.47–0.72, and $p < 0.001$), and pT3–4 disease (HR = 0.61, 95% CI: 0.52–0.73, and $p < 0.001$). Similar results were obtained from other meta-analyses (22, 23). A source of bias in the above studies was that the demographics of the patient populations were different and PLND was performed at the clinician's discretion. In a recent systematic review, the influence of PLND on perioperative and oncologic outcomes in patients undergoing RC for MIBC was assessed (7). Due to large heterogeneity between the studies the original meta-analysis planned by the authors was not possible. But the final results showed that any PLND is better than no PLND and extended template might improve oncologic outcomes compared to standard PLND. However, the benefit of

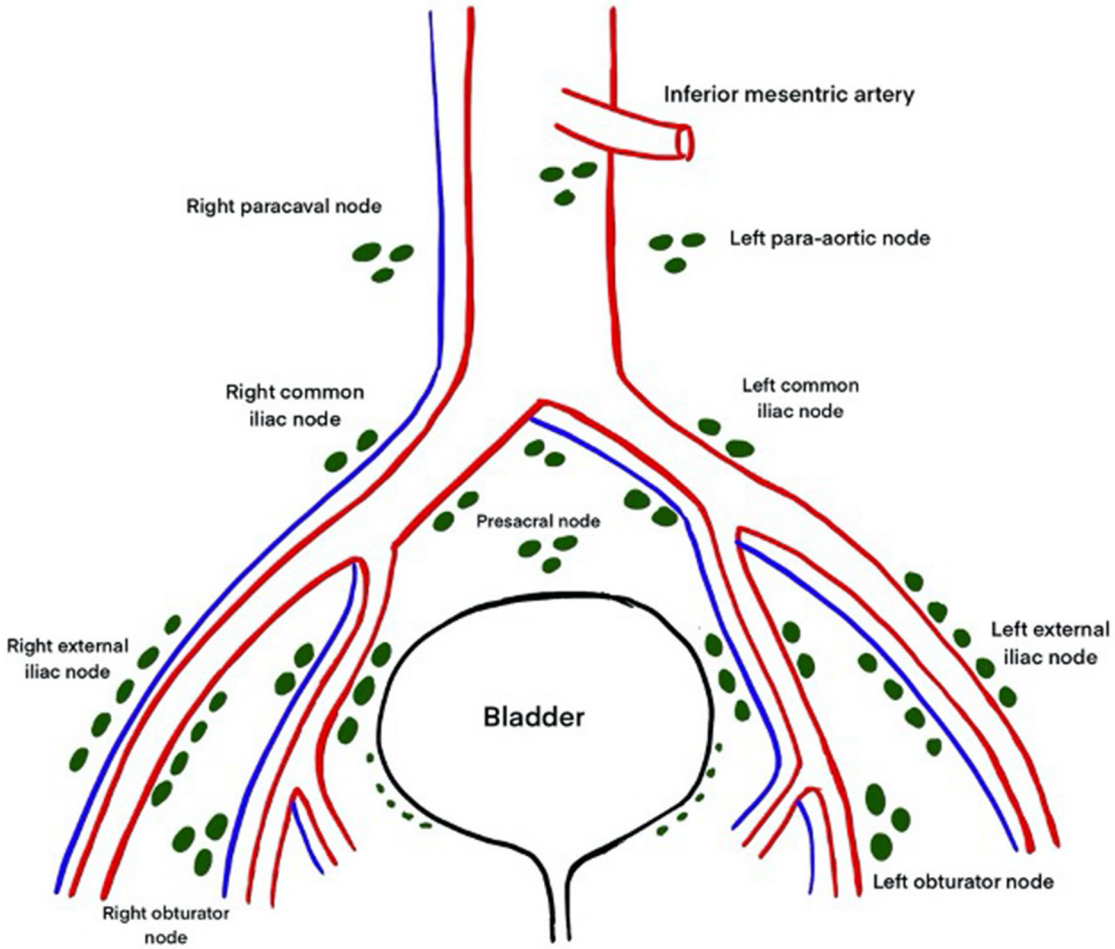

FIGURE 2 | Anatomy of lymphatic drainage of the bladder.

super-extended templates is unlikely when compared to extended template PLND.

Extended vs. Super-Extended PLND

Several groups published their results comparing extended PLND to super-extended PLND as mentioned in **Table 2**. Zehnder et al. (24) showed that super-extended PLND was not associated with a significantly improved 5-year RFS or OS even when stratified by node positivity. Møller et al. (25) did a similar comparison in 578 patients and reported no significant differences in RFS in the extended and super-extended groups. A trend toward increased RFS was seen in the >pT3 group in the super-extended cohort but was statistically insignificant. The younger age of patients in the super-extended cohort was also a potential source of bias. In light of the above evidence, super extended PLND appears to have no oncological benefit over extended PLND. This is probably because of the fact that metastatic spread beyond the anatomical pelvis could increase the risk of metastatic disease and further nodal deposits beyond the boundaries of the super-extended template.

Other Studies Comparing Various Lymph Node Dissection Templates

Holmer et al. (26) analyzed 69 and 101 patients under-going limited PLND (perivesical and obturator nodes) and standard PLND (limited regions plus the internal, external and common iliac nodes, and presacral nodes), respectively. They found no significant difference in DSS between the two groups. However, patients with pT3-4a disease were more common in the standard PLND group than in the limited one. Multivariate analysis revealed that there was significantly improved survival in the patients who underwent standard PLND, as shown in **Table 3**. Simone et al. (27) supported that extended PLND has both staging and therapeutic roles reporting better oncologic outcomes of patients who underwent extended PLND than standard PLND. They showed that patients who underwent an extended PLND had a significant improvement of disease-free survival (DFS) (HR = 1.96, 95% CI: 1.56–2.47, and $P < 0.001$) and CSS (HR = 1.76, 95% CI: 1.36–2.99, and $P < 0.001$) probabilities compared to s-PLND. Thus, they described a therapeutic result of extending PLND from the iliac bifurcation

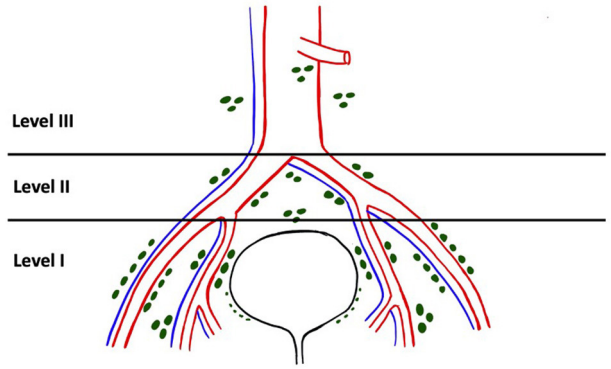

FIGURE 3 | Levels of lymph node dissection during radical cystectomy as described by Leissner et al.

TABLE 2 | Description of anatomical fields for extended PLND by Leissner et al.

Anatomical site	Boundaries (cranial—caudal—medial—lateral)
Right para-caval	Level of inferior mesenteric artery—aortic bifurcation—midline of vena cava—right ureter
Inter aortocaval	Level of inferior mesenteric artery—aortic bifurcation—midline of vena cava—midline of aorta
Left paraaortic	Level of inferior mesenteric artery—aortic bifurcation—midline of aorta—left ureter
Lateral to right common iliac artery	Aortic bifurcation—bifurcation of internal and external iliac arteries—midline of common iliac artery—psoas muscle
Lateral to left common iliac artery	Aortic bifurcation—bifurcation of internal and external iliac arteries—midline of common iliac artery—psoas muscle
Lateral to right external iliac artery	Bifurcation of internal and external iliac arteries—pelvic floor—midline of external iliac artery—genitofemoral nerve
Lateral to left external iliac artery	Bifurcation of internal and external iliac arteries—pelvic floor—midline of external iliac artery—genitofemoral nerve
Pre-sacral	Triangle between midline of the common iliac arteries—bifurcation of internal and external iliac arteries, dorsal border is sacrum
Right obturator space	Bifurcation of internal and external iliac arteries—pelvic floor—obturator nerve—midline of external iliac artery
Left obturator space	Bifurcation of internal and external iliac arteries—pelvic floor—obturator nerve—midline of external iliac artery
Right deep obturator space	Origin of the obturator nerve—pelvic floor—bladder wall—pelvic side wall
Left deep obturator space	Origin of the obturator nerve—pelvic floor—bladder wall—pelvic side wall

up to the aortic bifurcation. In a study by Abdi et al., extended PLND appeared to reduce the risk of local recurrence, but was not an independent predictor of overall survival (28). Extended PLND was associated with greater blood loss than s-PLND, but not with other perioperative complications. In contrast, Hugen et al. (29) compared standard and extended lymph node dissection and found no difference in 5-year recurrence free survival when stratified by node yield using the Kaplan–Meier method ($P = 0.138$). **Table 3** summarizes the conclusion drawn by various studies on the adequacy of pelvic lymph node dissection extent.

Lessons From the LEA and SWOG S1011 Trials (Table 3)

The above literature supporting extended PLND was somewhat challenged by the findings of the LEA trial, which is the first prospective randomized phase III trial comparing standard PLND with super-extended PLND (31). Extended LND ($n = 198$) failed to show a significant advantage over standard LND ($n = 203$) for RFS [5-year RFS 65 vs. 59%; hazard ratio 0.84; 95% confidence interval (CI): 0.58–1.22], cancer-specific survival (CSS) (5-year CSS 76 vs. 65%; HR 0.70; 95% CI 0.46–1.07) and OS (5-year OS 59 vs. 50%; HR 0.78; 95% CI: 0.57–1.07). However, a significant number of confounding factors are evident in this study. None of the patients received neoadjuvant chemotherapy. The relatively high percentage (14%) of pT1 disease could have limited the results' strength since more extensive PLND usually benefits those with more advanced disease. Post-operative chemotherapy was given at the discretion of the physician. Also, the relatively high percentage of positive surgical margins, 8.9% in the limited and 8.6% in the extended arm, raise questions about the adequacy of RC and if this could have influenced the OS and RFS. Lastly, this study was not powered to demonstrate the non-inferiority of standard PLND to super-extended PLND.

However, this trial has given us some valuable pointers. Firstly, 11% patients had positive lymph nodes located outside the standard PLND template in the super-extended group. If these patients had undergone a standard PLND, 2% would have had a false diagnosis of node negative disease. Also, of the total number of identified lymph nodes, 35% were solely in the super-extended template and would have been missed in a standard PLND. So, by using a super-extended, the authors identified 2% more patients as node positive and resected 35% more positive LNs, which would have been left behind using a standard PLND, with a limited increase in morbidity. In addition, the survival curves show some clinically significant but statistically insignificant divergence in respect to all the 3 endpoints and longer follow up is necessary. The results of the SWOG S1011 trial are awaited (32). This trial has a similar study design to the LEA trial, but it excludes patients with pT1 disease. Also 56% of patients in this trial received neoadjuvant therapy, hence it is more representative of the real world scenario. The accrual is complete and the estimated completion date is August 2022. **Table 4** compares chief characteristics of both of these trials.

Significance of Lymph Node Count, Lymph Node Density, and Extra-Nodal Extension

Lymph node count from a dissected specimen is influenced by various factors like method of lymph node submission (en-bloc vs. separate packets and the number of packets sent), surgical technique, and variability in the pathologic practices

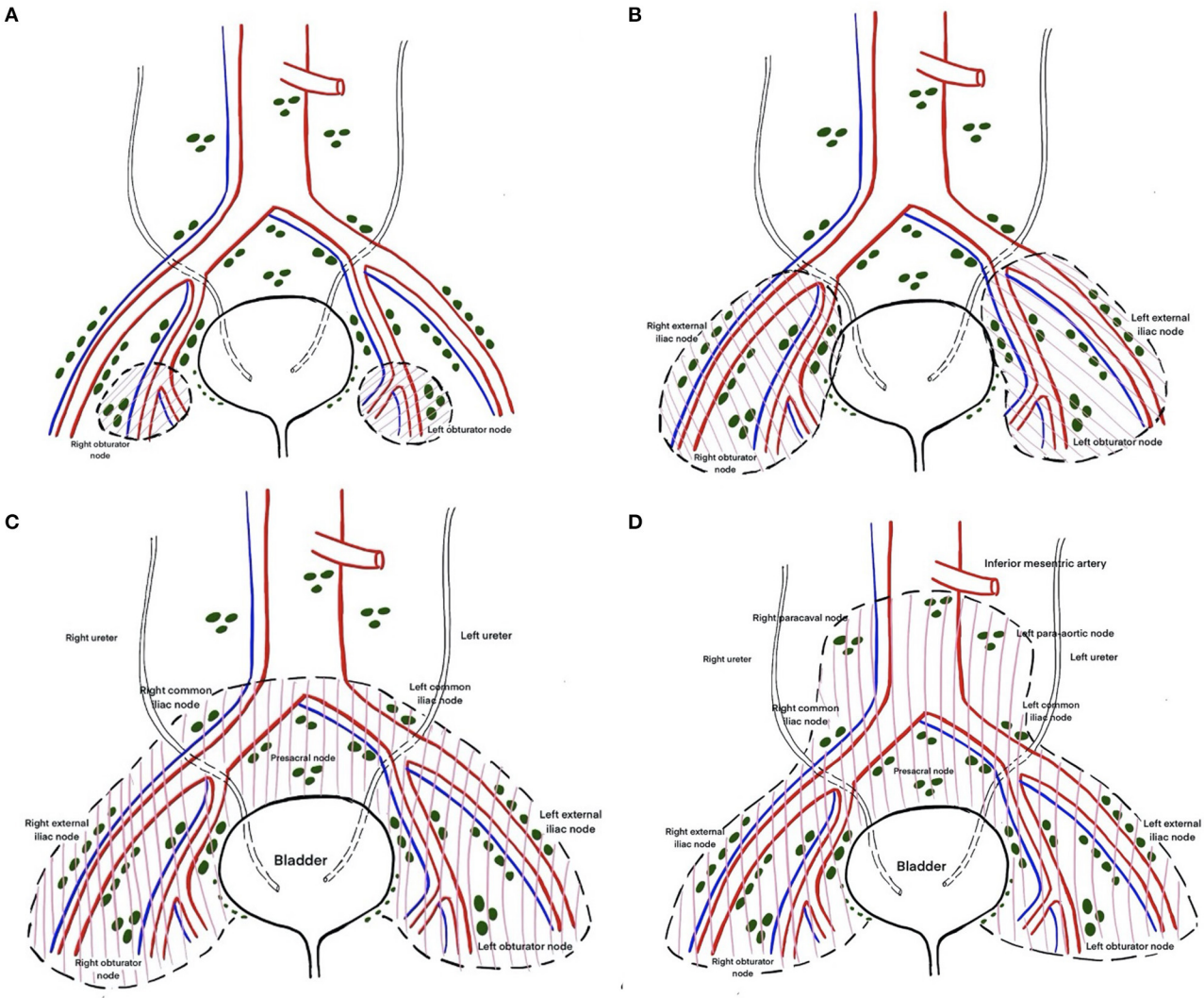

FIGURE 4 | (A) Limited lymph node dissection including only bilateral obturator and perivesical lymph nodes. **(B)** Standard template of lymph node dissection including lymph nodes of the external and internal iliac groups, up to bifurcation of the common iliac artery. **(C)** Extended template of lymph node dissection including lymph nodes up to the aortic bifurcation. **(D)** Super extended lymph node dissection including lymph nodes above the aortic bifurcation below the origin of the inferior mesenteric artery.

and reporting standards, along with inter individual variability in retrieving lymph nodes from the same template (33). Lymph node density refers to the ratio of positive lymph nodes on histopathology to the total number of nodes removed (29). Extra-nodal extension of tumor in an involved lymph node refers to the growth of a nodal cancer metastasis beyond the confines of the capsule of a lymph node into the adjacent tissues (34).

Multiple authors have reported the decreased probability of cancer death with increased number of lymph nodes harvested (35–39). The mechanism of this benefit is probably the removal of undetected micro-metastases, particularly in the setting of neoadjuvant or adjuvant therapies (40). Confounding factors like patient, surgeon, or institutional factors might also contribute to improved outcomes (41). Capitanio et al. (42) evaluated the probability of detecting node positive disease in a multi-institutional cohort of 731 patients based on total number of lymph nodes removed. 23.8% patients had positive nodes. Using receiver operating characteristic (ROC) curves, the authors predicted a 75% chance of identifying one or more lymph node metastases if 25 nodes were removed which improved to 90% with 45 nodes and decreased to 50% if 15–25 nodes were removed. So, a 25-node minimum was a reasonable cut-off to adequately stage and detect lymph node metastasis. In a retrospective analysis by Koppie et al. (43) from the MSKCC group, multivariate analysis showed that that increased lymph node counts did not correlate with increased survival above a count of 23. However, none of the authors could identify a

TABLE 3 | Comparison of lymph node yield in different PLND templates in various studies.

S.NO.	References	Year	Type of LND		Number of cases		Median lymph node yield		Primary end point	Conclusion
			Control group	Intervention group	Control group	Intervention group	Control group	Intervention group		
1	Bochner et al. (11)	2004	sLND	eLND	72	72	8	22	Staging advantage	No staging advantage was observed in eLND group as compared to sLND
2	Dhar et al. (19)	2008	sLND	eLND	336	322	12	22	RFS, OS	RFS 23 vs. 57% ($p < 0.0001$), OS 26 vs. 46% ($p = 0.0021$), in favor of the extended LND group
3	Zehnder et al. (24)	2011	eLND	seLND	405	554	22	38	RFS, OS	sePLND not associated with a significantly improved 5-year RFS or OS when stratified by node positivity
4	Holmer et al. (26)	2009	lLND	sLND	69	101	8	37	DSS	No significant difference in DSS
5	Simone et al. (27)	2012	sLND	eLND	584	349	18	29	DFS, CSS	e-PLND group had a significant improvement of DFS ($P < 0.001$) and CSS ($P < 0.001$) compared to s-PLND
6	Abdi et al. (28)	2016	sLND	eLND	105	105	9	21	RFS, OS	ePLND associated with a better local recurrence free survival (HR = 0.63, $P = 0.005$), but not an independent predictor of overall survival (HR = 1.06, $P = 0.84$)
7	Hugen et al. (29)	2010	sLND	eLND	206	54	9	46	RFS	No difference in 5-year RFS when stratified by node yield
8	Gschwend et al. (31)	2019	lLND	eLND	203	198	19	31	RFS, OS, CSS	eLND failed to show superiority over lLND with regard to RFS (5-year RFS 65 vs. 59%; hazard ratio [HR] = 0.84 [95% confidence interval 0.58–1.22]; $p = 0.36$), CSS (5-year CSS 76 vs. 65%; HR = 0.70; $p = 0.10$), and OS (5-year OS 59 vs. 50%; HR = 0.78; $p = 0.12$)

LND, lymph node dissection; lLND, limited LND; sLND, standard LND; eLND, extended LND; seLND, super-extended LND; RFS, recurrence free survival; OS, overall survival; DSS, disease specific survival; DFS, disease free survival; CSS, cancer specific survival.

continuous group of lymph nodes to be an independent predictor of cancer specific survival.

Increased lymph node density >20% decreases OS to <10% (41, 44–46). Extra-nodal extension of tumor is also a bad prognostic factor. A meta-analysis of 1,893 patients showed that it considerably correlated with reduced RFS (HR = 1.56, 95% CI: 1.13–2.14) and cancer-specific survival (HR = 1.60, 95% CI: 1.29–1.99) but not OS (HR = 1.47, 95% CI: 0.71–3.05) (47). **Figure 5** is a bar diagram representing minimum lymph node yield to be predictive of outcome of urinary bladder cancer as stated in different studies.

Prior to the publication of the LEA trial, majority of the retrospective and prospective literature were in favor of extended lymphadenectomy providing a survival advantage over standard lymphadenectomy. Many authors used factors like lymph node number, lymph density, extra nodal extension,

TABLE 4 | Comparison of the LEA and SWOG-1011 trial (30).

Characteristics	LEA	SWOG-1011
Identifier	NCT01215071	NCT01224665
Status	Completed	Ongoing
Comparing	sLND vs. seLND	sLND vs. seLND
Tumor stage	T1–T4a	T2–T4a
Primary endpoint	RFS at 5 years	RFS at 3 years

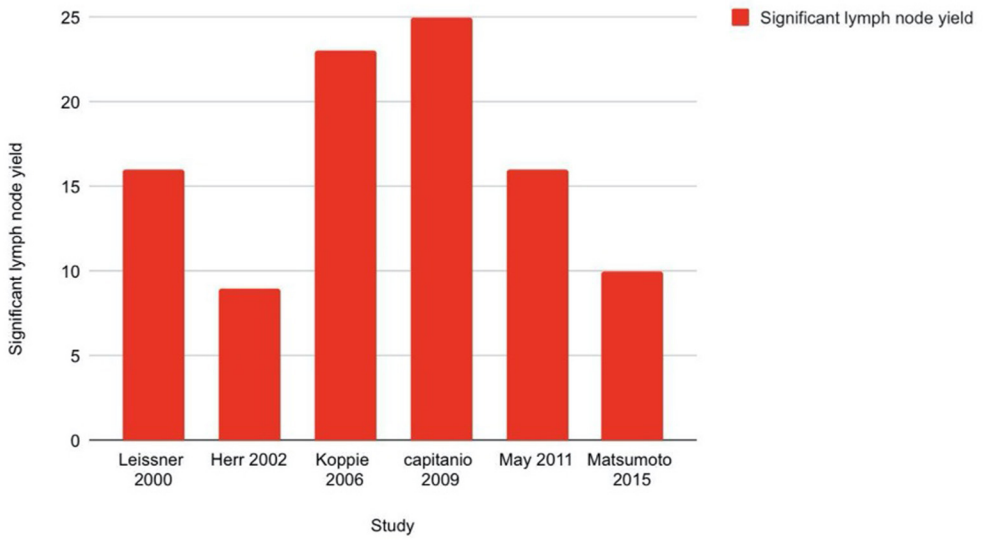

FIGURE 5 | Bar diagram showing minimum lymph node yield to be predictive of outcome of urinary bladder cancer.

etc., as markers of adequacy of lymphadenectomy and many studies showed significant correlation between number of lymph nodes dissected, lymph node density and oncological outcomes after radical cystectomy. Since extended lymphadenectomy was thought to harvest more lymph nodes and provide a better idea about lymph density it was recommended over and above standard dissection in these studies. Now, whether template is more important or number of harvested lymph nodes? A meticulous dissection with emphasis on skeletonization of the pelvic vessels is more appropriate than relying on the absolute lymph node count. There is a reasonable amount of consensus on this and that is why contemporary studies comparing various types of pelvic lymphadenectomy in bladder cancer make use of fixed anatomical templates rather than absolute number of lymph nodes.

Current Guidelines

Both the EAU and AUA guidelines recommend PLND during RC. However, they are guarded in elaborating the extent of lymphadenectomy required. The EAU guidelines state that "extended LND might have a therapeutic benefit compared to less extensive PLND, but due to bias, no firm conclusions can be drawn" (48). The NCCN guidelines recommend that bilateral PLND should be performed, with a minimum of common iliac, internal iliac, and obturator nodes excised (49). It also states that a more extensive PLND, which may include the common iliac and the lower para-aortic and paracaval nodes may be associated with better survival and lower local recurrence rates. Still, it stops short of recommending this type of PLND. The AUA guidelines state that a bilateral PLND should be performed in every surgery with a curative intent (grade B). The standard PLND template with a minimum total lymph node count of 12 should be included (50). Even though one prospective randomized study has been published, the lack of robust clinical data on the various extents of PLND probably limits the scope of recommendations in the above guidelines.

CONCLUSION AND RECOMMENDATIONS

None of the surrogate markers of adequacy of PLND, namely anatomical template, lymph node number or lymph node density have been recommended forthright due to the lack of robust prospective data. In even the most recent meta-analysis on this topic, most of the included studies were retrospective. The differences in use of neoadjuvant chemotherapy worldwide also make it difficult to compare the studies without introducing bias. The extent of lymphadenectomy was left at the surgeon's discretion in most retrospective studies, thereby lacking randomization. Therefore, all of these issues should be accounted for before attempting to compare the different extents of PLND, reported in different studies. Therefore, to facilitate the above, based on this review, we wish to emphasize upon and recommend the following points:

- Level of pelvic lymph nodes are used to denote specific anatomical locations of the pelvic lymph nodes. The extent of pelvic lymphadenectomy would be denoted by whether just one, two, or all three levels of lymph nodes have been dissected out during PLND.
- Anatomical templates of dissection have been defined in the preceding sections and this nomenclature should be strictly followed in all future trials to bring uniformity and facilitate comparison.
- Whenever possible, we should adhere to guidelines and try to do an extended PLND, especially whenever there is visible lymphadenopathy on exploration and imaging.

- Super-extended PLND appears to have no survival benefit over extended PLND and its use should be decided on a case to case basis.
- PLND should be deemed adequate if an anatomical template is followed and thorough dissection is done rather than relying on the number of harvested lymph nodes or the lymph node density. Anatomical recommendations of a particular template are favorable and generalizable to a wider clinical community. This obviates the reliance on histopathologist for lymph node identification and subsequent prognostication.

AUTHOR CONTRIBUTIONS

RJ and NS have contributed equally to writing and manuscript preparation of this work. RJ and APS have conceived the idea if the review and have done the search strategy and selection of the articles and have contributed equally to the process of review and critical analysis. GC and AS have helped equally in the selection of articles for review and in final revision and manuscript preparation. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Surgery

ORIGINAL RESEARCH
published: 23 August 2021
doi: 10.3389/fsurg.2021.704902

T1 Bladder Cancer: Comparison of the Prognostic Impact of Two Substaging Systems on Disease Recurrence and Progression and Suggestion of a Novel Nomogram

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Edited by:
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Reviewed by:
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Marcelo Langer Wroclawski, Albert Einstein Institute for Teaching and Research (IIEP), Brazil

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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *04 May 2021*
Accepted: *19 July 2021*
Published: *23 August 2021*

Citation:
Asimakopoulos AD, Colalillo G, Telesca R, Mauriello A, Miano R, Di Stasi SM, Germani S, Finazzi Agrò E, Petrozza V, Caruso G, Carbone A, Pastore AL and Fuschi A (2021) T1 Bladder Cancer: Comparison of the Prognostic Impact of Two Substaging Systems on Disease Recurrence and Progression and Suggestion of a Novel Nomogram. Front. Surg. 8:704902. doi: 10.3389/fsurg.2021.704902

Background: The T1 substaging of bladder cancer (BCa) potentially impacts disease progression. The objective of the study was to compare the prognostic accuracy of two substaging systems on the recurrence and progression of primary pathologic T1 (pT1) BCa and to test a nomogram based on pT1 substaging for predicting recurrence-free survival (RFS) and progression-free survival (PFS).

Methods: The medical records of 204 patients affected by pT1 BCa were retrospectively reviewed. Substaging was defined according to the depth of lamina propria invasion in $T1_{a-c}$ and the extension of the lamina propria invasion to T1-microinvasive ($T1_m$) or T1-extensive ($T1_e$). Uni- and multivariable Cox regression models evaluated the independent variables correlated with recurrence and progression. The predictive accuracies of the two substaging systems were compared by Harrell's C index. Multivariate Cox regression models for the RFS and PFS were also depicted by a nomogram.

Results: The 5-year RFS was 47.5% with a significant difference between $T1_c$ and $T1_a$ ($p = 0.02$) and between $T1_e$ and $T1_m$ ($p < 0.001$). The 5-year PFS was 75.9% with a significant difference between $T1_c$ and $T1_a$ ($p = 0.011$) and between $T1_e$ and $T1_m$ ($p < 0.001$). Model $T1_{m-e}$ showed a higher predictive power than $T1_{a-c}$ for predicting RFS and PFS. In the univariate and multivariate model subcategory T1e, the diameter, location, and number of tumors were confirmed as factors influencing recurrence and progression after adjusting for the other variables. The nomogram incorporating the $T1_{m-e}$ model showed a satisfactory agreement between model predictions at 5 years and actual observations.

Conclusions: Substaging is significantly associated with RFS and PFS for patients affected by T1 BCa and should be included in innovative prognostic nomograms.

Keywords: bladder cancer, urothelial carcinoma, staging, lamina propria, prognosis, urinary bladder neoplasms

INTRODUCTION

Up to 80% of bladder cancers (BCa) are non–muscle invasive during the first diagnosis (1); ∼25% present as T1 lesions with invasion of the subepithelial connective tissue (i.e., lamina propria) (1–3).

The management of T1 BCa is controversial since it presents different degrees of aggressiveness, with a progression rate varying from 12 to 54% (4, 5). Many clinical (age, gender, multifocality, and tumor size) and pathologic (concomitant CIS, tumor grade, and architecture) factors for recurrence and progression have been extensively studied over the years to predict prognosis and guide management decisions (1, 6).

Younes et al. (7) were the first to use the muscularis mucosa (MM) in transurethrally resected biopsy specimens to substage (i.e., subcategorize) T1 BCa. Since then, several studies have been conducted to identify whether the depth of lamina propria invasion is a valuable prognostic factor with the use of the MM as a landmark for T1 substaging. This was done according to the location of the invasion: above the muscularis mucosa–vascular plexus (MM-VP) ($T1_a$), in the MM-VP ($T1_b$), or beyond the MM-VP ($T1_c$). A recent meta-analysis demonstrated a worse prognosis for T1 tumors with deep MM invasion (8). However, other studies underlined that the substaging of T1 BCa is technically difficult because MM and VP represent inconsistent histologic landmarks for staging (i.e., not always present). Furthermore, if present, these structures can be overrun by the invasive tumor, making substaging challenging. In fact, a lack of consensus among pathologists regarding the identification of the MM-VP at the invasion front of the tumor has been reported, while others suggest that the T1 substaging does not add prognostic value with respect to survival (9, 10).

In 2009, van Rhijn (1) evaluated another, more "user-friendly" method intended as a more feasible substaging system that does not require the identification of the MM-VP to subcategorize T1 BCa and discern between T1-microinvasive ($T1_m$) and

TABLE 1 | Clinical and demographic characteristics.

Patients		N	204
Age, years		mean (SD)	72 (9.5)
Sex	M	n (%)	180 (87%)
Smoking	Yes	n (%)	85 (41.7%)
Neutrophil/lymphocyte, $n = 191$		Median (25–75 percentile)	4 (2.37–6.36)
Trigone/bladder neck	Yes	*n* (%)	65 (30.9%)
Tumor diameter, $N = 203$	>3 cm	*n* (%)	43 (21.2%)
N of tumors	1	*n* (%)	106 (52%)
	2–7	*n* (%)	88 (43.1%)
	≥8	*n* (%)	10 (4.9%)
Substage T1a-c	T1a	*n* (%)	97 (47.6%)
	T1b	*n* (%)	49 (24%)
	T1c	*n* (%)	39 (19.1%)
	Not evaluable (NV)	*n* (%)	19 (9.3%)
Substage T1m-e	T1m	*n* (%)	90 (44.1%)
	T1e	*n* (%)	79 (38.7%)
	Not evaluable (NV)	*n* (%)	35 (17.2%)
Grade	G1	*n* (%)	28 (13.7%)
	G2	*n* (%)	1 (0.5%)
	G3	*n* (%)	175 (85.8%)
CIS	Yes	*n* (%)	5 (2.5%)
Muscolaris propria	Yes	*n* (%)	167 (81.9%)
LVI, $n = 203$	Yes	*n* (%)	24 (11.8%)
Tumor pattern	Solid	*n* (%)	2 (1%)
Squamous metaplasia	Yes	n (%)	8 (3.9%)
Re-turb within 4 weeks, $n = 203$	Yes	*n* (%)	41 (20.2%)
BCG instillations, $n = 202$	Yes	*n* (%)	140 (70.4%)
Follow up, months		Median (25–75 percentile)	37.5 (21 –51.5)

N, number; SD, standard deviation; M, male; CIS, carcinoma in situ; LVI, lymphovascular invasion; TURB, transurethral resection of bladder; BCG, bacillus Calmette-Guérin.
Not evaluable (NV) =T1 cases of bladder cancer where substaging was not feasible for several reasons such as.
-artifacts of the microscope slides.
-absence of the muscularis mucosae or its thinness, fragmentation and/or coagulation related to TUR.
-practical difficulties in defining the level of tumor invasion according to the orientation of specimens.

TABLE 2 | Recurrence, progression and mortality in the current series.

Patients	N	204
Recurrence	*n* (%)	106 (52%)
Progression	*n* (%)	45 (22.1%)
Radical cystectomy, $n = 202$	*n* (%)	35 (17.3%)
Cancer specific mortality, $n = 165$	*n* (%)	14 (8.5%)
Death for other cause, $n = 156$	*n* (%)	13 (8.3%)

T1-extensive-invasive ($T1_e$) tumors, as described by van der Aa et al. (11). In their multivariable analyses, substage ($T1_{m-e}$) was significant for progression and disease specific survival, whereas substage according to $T1_{a-c}$ was not significant.

The objective of the study was to evaluate and compare the prognostic significance of two substaging systems ($pT1_{a-c}$ and $pT1_{m-e}$) on the rate of recurrence and progression of the T1 BCa. A novel nomogram that incorporates T1 substaging and depicts the probability of recurrence and progression-free survival was also created.

MATERIALS AND METHODS

Between 2009 and 2017, 787 patients underwent a transurethral resection (TUR) for BCa at two institutions; 240 of them were diagnosed as having primary pathologic T1 (pT1) urothelial BCa. Out of 240 patients, 30 were excluded for incomplete data while six were excluded because of concomitant urothelial carcinoma in the upper urinary tract. Consequently, the medical records of 204 patients were retrospectively reviewed.

Random biopsies, a standard repeat transurethral resection (re-TUR), and a single instillation of chemotherapy after TUR were not routinely performed. The surveillance of the patients consisted of cystoscopy and cytology every 3–4 months in the first 2 years and subsequently at a lower frequency (6–12 months) if no recurrence was detected. Upper urinary tract imaging was done every 1–2 years or when indicated by clinical suspicion.

Available data regarding gender, age, smoking, neutrophil to lymphocyte ratio, bladder neck or trigonal location of the tumor, tumor diameter $<$ or >3 cm, number of tumors (1 vs. 2–7 vs. >8), grade, concomitant carcinoma *in situ* (Cis), presence of muscularis propria, lymphovascular invasion (LVI), tumor pattern (papillary vs. solid), presence of associated squamous metaplasia, performance of re-TUR, Bacillus Calmette-Guerin (BCG) instillations, recurrence and time to recurrence, progression and time to progression, performance of radical cystectomy, cancer-specific mortality, death for other causes, and follow-up calculated in months until the last clinical office visit or death was inserted in a customized, institutional review board-approved database (PTV registration number 255.19). Pathology information was recorded from the pathology report but was fully reevaluated as part of the study.

Recurrence was defined as the histological detection of BCa through transurethral bladder resection (TUR) or bladder biopsy following the first TUR or the re-TUR. Progression was defined as the development of muscle-invasive disease or distant metastasis.

The TUR specimens of each institution were reviewed by their respective dedicated uropathologists. Tumors were staged according to the recently published American Joint Committee on Cancer (AJCC) Staging Manual 8th edition (12). The World Health Organization (WHO) 1973 classification system for grade was used to determine a grade, since Pellucchi et al. demonstrated that this grading system better stratifies patients with lamina propria invasion (13). Thus, T1 substaging was defined according to the depth of lamina propria invasion using the Younes et al. (7) classification as follows: $T1_a$, invasion of the lamina propria superficial to the level of the MM; $T1_b$, invasion to the level of the MM; $T1_c$, invasion through the level of the MM but superficial to the muscularis propria. If the MM-VP was not present at the invasion front, the case was assigned to $T1_a$ or $T1_c$ based on the extent of invasion into the lamina propria by looking at the MM-VP in tumor-free areas in the same or other TUR chips. Otherwise, if the associated vascular plexus could be identified, then it served as a marker for the MM level.

The definition of $T1_m$ was a single focus of lamina propria invasion with a diameter of $<$0.5 mm (within one high-power field, objective $\times$ 40). Specimens showing a larger area with invasion or multiple microinvasive areas were considered $T1_e$ (1).

Substages $T1_{a-c}$ and $T1_{m-e}$ were then inserted into the customized database.

Statistical Analysis

Categorical variables were described in terms of frequency (*n*) and percentage (%). Continuous variables were described as mean and SD or, if not normally distributed, as median and interquartile range (from the 25th to 75th percentile).

The association between categorical variables was tested by chi-square or, when appropriate, by Fisher's exact test. Differences in continuous variables among more than two groups were tested with the Kruskal–Wallis test. Post-comparisons were performed applying the Mann–Whitney test.

A log-rank test was applied to compare the survival curves among the substages. Uni- and multivariable Cox regression models were performed to evaluate the independent variables that influence recurrence-free survival (RFS). Variables with a $p < 0.1$ at the univariable analysis were considered in the multivariable model. Due to the low number of events in the model on progression-free survival (PFS), a stepwise selection method was applied to select the best set of predictors.

To compare the prognostic performance of the two substaging systems ($T1_{a-c}$-Model 1 vs. $T1_{m-e}$-Model 2), a univariable cox regression model was performed; then the prediction accuracy of these two models was compared by comparing the Harrell's C indexes while Lincom Stata command was applied to test the difference between Harrell's C.

The multivariate Cox regression model for RFS and PFS was depicted by a nomogram, considering the probability of survival at 5 years. In the nomogram, we assigned a score from 0 to 100 corresponding to the value of each predictor. By adding the singular scores, a total score was obtained that corresponded to the 5-year survival probability.

TABLE 3 | Association between Subcategory T1a-c and other characteristics.

		Substage					
		T1a ***n* = 97**	**T1b** ***n* = 49**	**T1c** ***n* = 39**	**NV** ***n* = 19**	***p***	**B-H adjusted *p***
Smoke						**0.001**	**0.002**
Yes	*n* (%)	36 (37.1%)	14 (28.6%)	27 (69.2%)	8 (42.1%)		
Neutrophil/lymphocyte							
	Median (25–75 percentile)	3.8 (2.11–5.8)	4.34 (2.59–6.3)	5.8 (3.7–7.6)	1.65 (1.28–2.86)	**0.0001**[a]	**0.001**
Trigone/bladder neck						**0.001**	**0.002**
Yes	*n* (%)	30 (30.9%)	6 (12.2%)	20 (51.3%)	7 (36.8%)		
Tumor diameter, *N* = 203						**<0.001**	**0.002**
>3 cm	*n* (%)	16 (16.7%)	6 (12.2%)	18 (46.2%)	3 (15.8%)		
***N* of tumors**						0.594	0.594
1	*n* (%)	46 (47.4%)	29 (59.2%)	18 (46.2%)	13 (68.4%)		
2–7	*n* (%)	46 (47.4%)	18 (36.7%)	19 (48.7%)	5 (26.3%)		
≥8	*n* (%)	5 (5.2%)	2 (4.1%)	2 (5.1%)	1 (5.3%)		
Grade						**0.003***	**0.006**
G1	*n* (%)	21 (21.7%)	4 (8.2%)	0 (0%)	3 (15.8%)		
G2	*n* (%)	1 (1%)	0 (0%)	0 (0%)	0 (0%)		
G3	*n* (%)	75 (77.3%)	45 (91.8%)	39 (100%)	16 (84.2%)		
Cis concomintant						**0.02***	**0.031**
Yes	*n* (%)	1 (1%)	0 (0%)	4 (10.3%)	0 (0%)		
Muscolaris propria						**0.019**	**0.031**
Yes	*n* (%)	76 (78.4%)	38 (77.6%)	38 (97.4%)	15 (79%)		
Lymphovascular invasion						**<0.001**	**0.001**
Yes	*n* (%)	4 (4.2%)	5 (10.2%)	14 (35.9%)	1 (5.3%)		
Tumor pattern						0.363	0.391
Papillary	*n* (%)	97 (100%)	48 (98%)	38 (97.4%)	19 (100%)		
Squamous metaplasia, *N* = 203						0.323	0.377
Yes	*n* (%)	5 (5.2%)	0 (0%)	2 (5.1%)	1 (5.3%)		
Re-turb, *N* = 203						0.055	0.077
Yes	*n* (%)	19 (19.8%)	12 (24.5%)	3 (7.7%)	7 (36.8%)		
BCG, *N* = 199						0.073	0.093
Yes	*n* (%)	64 (68.1%)	30 (63.8%)	34 (87.2%)	12 (63.2%)		
Radical cystectomy, *N* = 202						**<0.001**	**0.001**
Yes	*n* (%)	10 (10.3%)	7 (14.6%)	16 (41%)	2 (11.1%)		

NV, not evaluable; B-H, Benjamini-Hockberg.

**Fisher exact test.*

[a]*Kruskal Wallis test. Post hoc comparisons showed: Non significant difference between T1a vs T1b (p = 0.314); Significant difference between T1a vs T1c (p = 0.006), vs NV (p = 0.007); Non significant difference between T1b vs T1c (p = 0.056), significant difference between T1b vs NV (p = 0.006); Significant difference between T1c vs NV (p = 0.001). All p values were adjusted for multiple comparisons (Benjamini-Hockberg method).*

Bold indicate the p value <0.05.

The nomogram was subjected to bootstrap resamples for the reduction of overfit bias of predicted vs. observed values depicted in the calibration plot. For the Cox models, the predicted value was obtained at a 5-year time-point while the observed value was the corresponding Kaplan–Meier survival estimate.

Statistical analysis was performed using the STATA (version 16.1) and R software (version 3.3.4, Regression Modeling Strategies package. The R Foundation for Statistical Computing, Vienna, Austria).

In multiple comparisons, the Benjamini–Hochberg method was applied to adjust the p values. A $p < 0.05$ was considered statistically significant.

RESULTS

The demographic and clinicopathological data were retrospectively collected for 204 patients (**Table 1**).

With a median follow-up of 37.5 months (IQR 21–51.5), 106 patients experienced a recurrence, and 45 showed progression of

TABLE 4 | Association between subcategory T1m-e and other characteristics.

		Substage T1m-e				
		T1m ***n* = 90**	**T1e** ***n* = 79**	**NV** ***n* = 35**	***p***	**B-H adjusted *p***
Smoke					0.766	0.766
Yes	*n* (%)	35 (38.9%)	35 (44.3%)	15 (42.9%)		
Neutrophil/lymphocyte						
	Median (25-75 percentile)	4 (2.76 – 6.36)	3.7 (2.02 – 6)	4.75 (1.64 – 7.3)	0.262[a]	0.435
Trigone/bladder neck					0.11	0.257
Yes	*n* (%)	22 (24.4%)	31 (39.2%)	10 (28.6%)		
Tumor diameter, *N* = 203					**<0.001**	**0.001**
>3 cm	*n* (%)	11 (12.4%)	28 (35.4%)	4 (11.4%)		
***N* of tumors**					0.24	0.435
1	*n* (%)	49 (54.4%)	34 (43%)	23 (65.7%)		
2-7	*n* (%)	37 (41.1%)	40 (50.6%)	11 (31.4%)		
≥8	*n* (%)	4 (4.4%)	5 (6.3%)	1 (2.9%)		
Grade					**0.040***	0.112
G1	*n* (%)	18 (20%)	5 (6.3%)	5 (14.3%)		
G2	*n* (%)	1 (1.1%)	0 (0%)	0 (0%)		
G3	*n* (%)	71 (78.9%)	74 (93.7%)	30 (85.7%)		
Cis concomintant					**0.019***	0.067
Yes	*n* (%)	0 (0%)	5 (6.3%)	0 (0%)		
Muscolaris propria					0.432	0.550
Yes	*n* (%)	74 (82.2%)	62 (78.5%)	31 (88.6%)		
Lymphovascular invasion					**<0.001**	**0.001**
Yes	*n* (%)	4 (4.4%)	18 (23.1%)	2 (5.7%)		
Tumor pattern					0.311*	0.435
Papillary	*n* (%)	90 (100%)	77 (97.5%)	35 (100%)		
Squamous metaplasia, *N* = 203						
Yes	*n* (%)	5 (5.6%)	2 (2.5%)	1 (2.9%)	0.71	0.765
Re-turb, N = 203						
Yes	*n* (%)	15 (16.9%)	18 (22.8%)	8 (22.9%)	0.577	0.673
BCG instillations, *N* = 199						
Yes	*n* (%)	57 (64.8%)	57 (75%)	26 (74.3%)	0.307	0.435
Radical cystectomy, *N* = 202						
Yes	*n* (%)	8 (8.9%)	25 (32.1%)	2 (5.9%)	**<0.001**	**0.001**

NV, not evaluable.
**Fisher exact test. [a]Kruskal Wallis test.*
B-H, Benjamini-Hockberg method to adjust p values for multiple comparisons.
Bold indicate the p value <0.05.

the disease (**Table 2**). The association between substages $T1_{a-c}$ and other clinical and demographic characteristics is shown in **Table 3**, while the association between substages $T1_{m-e}$ and other variables is shown in **Table 4**.

Among the subcategories $T1_{a-c}$, $T1_c$ had a significantly higher rate of both recurrence (71.8%) and progression (38.5%). Among the subcategories $T1_{m-e}$, $T1_e$ had a significantly higher rate of recurrence (70.9%) and progression (39.2%) (**Table 5**).

Recurrence-Free Survival

The 5-year RFS was 47.5% (SE = 3.6%, **Figure 1**). The mean and median RFS was about 3.99 and 1.75 years, respectively. The RFS per substage is shown in **Figure 2**.

Harrell's C of model 1 (M1) was equal to 0.563, while Harrell's C of model 2 (M2) was 0.616. Comparing the predictive power, the M2 model seemed to have a slightly higher predictive power (difference = 0.053; $p = 0.033$).

Uni- and multivariable Cox regression models were performed to evaluate which independent variables influence recurrence (**Table 6**). Substages $T1_{a-c}$ and $T1_{m-e}$ were highly associated; however, since, in the comparison between models, M2 showed a higher predictive power, it was the only one considered in the multivariable analysis. This analysis was depicted by nomogram 1 (**Figure 3**). The calibration plot was depicted in **Figure 4**, showing a satisfactory agreement between model predictions at 5 years and actual observations.

TABLE 5 | Recurrence and progression by subcategory.

	Substage T1a-c				
	T1a	**T1b**	**T1c**	**NV**	
Recurrence	45 (46.39%)	25 (51.02%)	28 (71.79%)	8 (42.11%)	0.044
Progression	16 (16.49%)	11 (22.45%)	15 (38.46%)	3 (15.79%)	0.040
	Substage T1m-e				
	T1m	**T1e**		**NV**	
Recurrence	40 (44.44%)	56 (70.89%)		10 (28.57%)	<0.001
Progression	11 (12.22%)	31 (39.24%)		3 (8.57%)	<0.001

Progression-Free Survival

The 5-year PFS was 75.9% (SE = 3.5%, **Figure 5**). The PFS median time was not evaluable because the survival curve did not reach 50%; in fact, the overall PFS was higher than 50%. Overall mean PFS time was 6.2 years.

The log-rank test revealed a significant difference in PFS ($p = 0.025$; **Figure 6A**); a significant difference in PFS was observed between $T1_c$ and $T1_a$ (log-rank adjusted $p = 0.011$) but not between $T1_c$ and $T1_b$ (log-rank adjusted $p = 0.415$). A significant difference in PFS was observed between $T1_e$ and $T1_m$ (**Figure 6B**; Log rank test $p < 0.001$).

Harrell's C of M1 was equal to 0.612 and 0.70 for M2. The M2 model showed a slightly higher predictive power than the M1 model (difference = 0.09; $p = 0.015$). Thus, only M2 was considered in the multivariable analysis (**Table 7**) that was depicted by nomogram 2 (**Figure 7**).

The calibration curve is depicted in **Figure 8**, showing a good agreement between model predictions at 5 years and actual observations.

The BCG showed a significant impact on prolonging PFS only for T1a patients ($p = 0.032$).

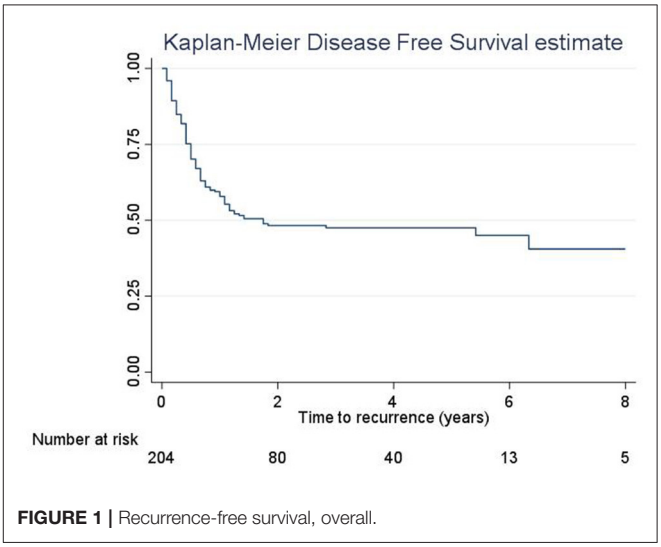

FIGURE 1 | Recurrence-free survival, overall.

DISCUSSION

The disease T1 BCa is heterogeneous. Although classified as non-muscle-invasive, it is potentially the most aggressive subtype of non-muscle invasive bladder cancer (NMIBC), with high rates of recurrence and considerable rates of progression (4, 5).

The European Organization for Research and Treatment of Cancer (EORTC) risk tables (6) and the Spanish Urological Club for Oncological Treatment (CUETO) scoring model (14) are the two best established predictive tools ("risk calculators") to help decision-making for patients with NMIBC. However, recent studies that assessed the performance of these predictive tools documented a poor discrimination for both disease recurrence and progression in NMIBC patients, with a tendency of these models to overestimate the risk of disease recurrence and progression in high-risk patients (15). These overestimations remained in BCG-treated patients, especially for the EORTC tables (15).

This overestimated risk may lead to overtreatment with early radical cystectomy for patients with T1 BCa. However, the suboptimal prognostic accuracy of the aforementioned risk calculators may be also associated with the risk of undertreatment, i.e., delays in receiving radical surgery. Consequently, these risk stratification tools may be implemented with new features that could improve their prognostic accuracy and allow for aligning therapy with the real clinical behavior of the individual tumor.

There is growing evidence that tumor depth invasion of the muscolaris mucosae as well as extensive or multifocal invasion of the lamina propria could be such a feature for patients with T1 BCa. A recent meta-analysis evaluated the prognostic value of the subcategorization of oncological outcomes in patients with T1 BCa (8). The prognostic value of pT1 substaging on at least one oncological outcome was established in 29 studies. In seven studies, with a total of 899 patients, MM invasion was associated with a higher disease progression rate (pooled HR of 2.61, 95% CI: 1.61–4.23) (5, 16–21). In six studies, with a total of 930 patients, MM invasion was associated with a higher disease recurrence rate (pooled HR of 1.23, 95% CI: 1.01–1.49)

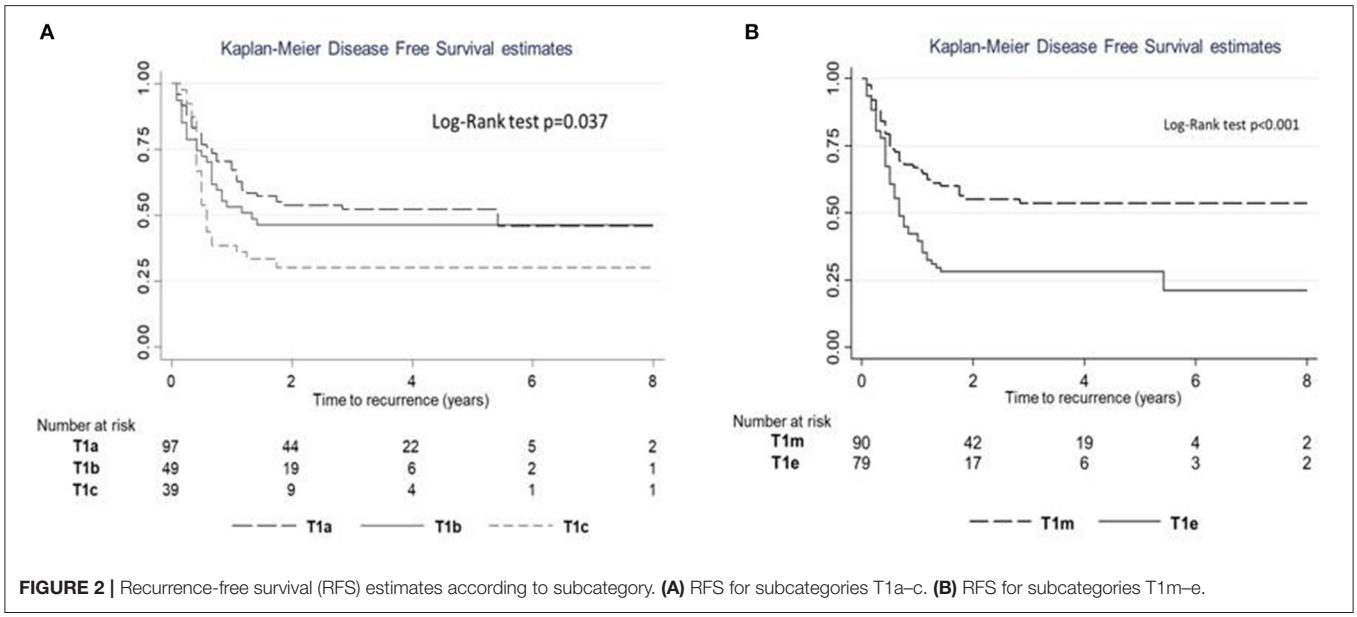

FIGURE 2 | Recurrence-free survival (RFS) estimates according to subcategory. **(A)** RFS for subcategories T1a–c. **(B)** RFS for subcategories T1m–e.

TABLE 6 | Uni and multivariable Cox regression model was performed to evaluate which independent variables influence the recurrence.

		Univariable				Multivariable*			
			95% CI				95% CI		
		HR	LL	UL	p	HR	LL	UL	p
Age, yrs		1	0.98	1.02	0.95				
Sex	M vs. F	0.95	0.53	1.7	0.873				
Smoking		1.1	0.75	1.63	0.616				
Neutrophil/lymphocyte, *n* = 191		0.93	0.85	1.01	**0.074**				
Trigone/bladder neck		1.71	1.15	2.54	**0.008**	1.5	0.99	2.27	0.055
Tumor diameter, *N*=203									
	>3 cm vs. <3 cm	1.93	1.25	2.98	**0.003**	1.7	1.05	2.75	0.032
N of tumors									
	2-7 vs. 1	1.7	1.13	2.55	**0.01**	1.75	1.13	2.7	0.012
	≥8 vs. 1	4.84	2.33	10.06	**<0.001**	3.68	1.66	8.19	0.001
Substage T1a-c									
	T1b vs. T1a	1.24	0.76	2.02	0.393	Not in the model			
	T1c vs. T1a	1.8	1.12	2.91	0.016				
	Not evaluable vs. T1a	0.9	0.41	2.01	0.802				
Substage T1m-e									
	T1e vs. T1m	2.03	1.35	3.07	0.001	1.59	1.02	2.46	0.039
	Not evaluable vs. T1m	0.544	0.26	1.12	0.1	0.57	0.28	1.19	0.136
Grade									
	G3 vs. G1	1.03	0.59	1.81	0.921				
CIS		1.93	0.71	5.26	0.2				
Muscolaris propria present		0.73	0.45	1.17	0.19				
Lymphovascular invasion, *n* = 203		1.62	0.96	2.74	**0.069**				
Tumor pattern	Solid	1.24	0.17	8.91	0.831				
Squamous metaplasia	Yes vs. no	2.31	1.01	5.29	**0.048**	1.39	0.55	3.51	0.482
BCG instillations *n* = 199	Yes	1.4	0.87	2.25	0.162				

Bold indicate the p value <0.10.

**N = 203.*

In the final model the following variables were excluded: LVI because it was highly correlated with Substaging T1m-e and Neutrophil/lymphocyte in order to avoid a significant reduction of the sample size.

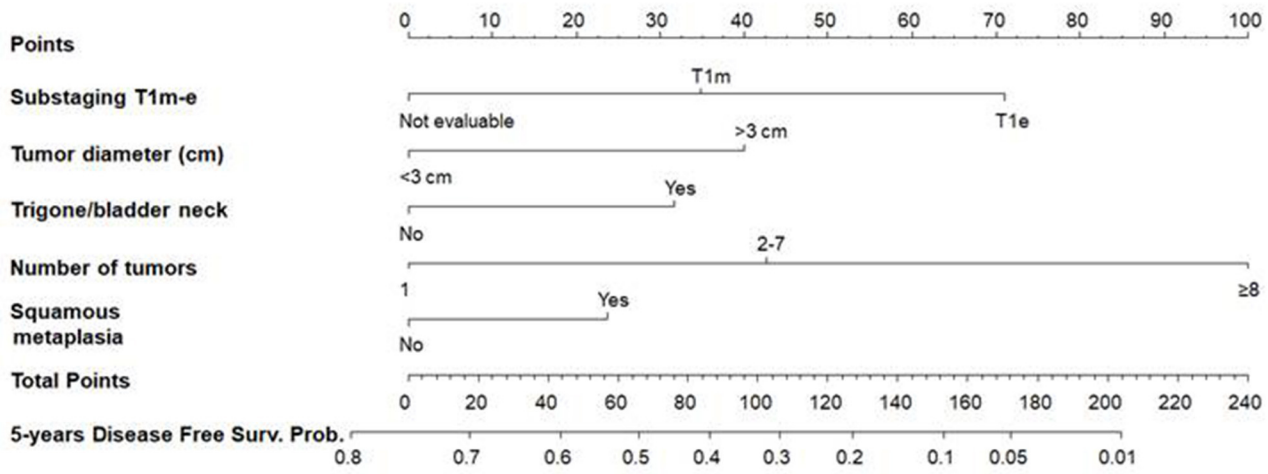

FIGURE 3 | Nomogram 1 for the calculation of the recurrence-free survival (RFS) at 5 years. On the basis of the estimated Cox regression model, patients with T1e (71 points), a >3 cm tumor diameter (40 points), trigone or bladder neck location (32 points), with a number of tumors ≥8 (100 points), and presence of squamous metaplasia (24) had a total score of 267, which corresponds to a low 5-year RFS probability ($p < 0.01$).

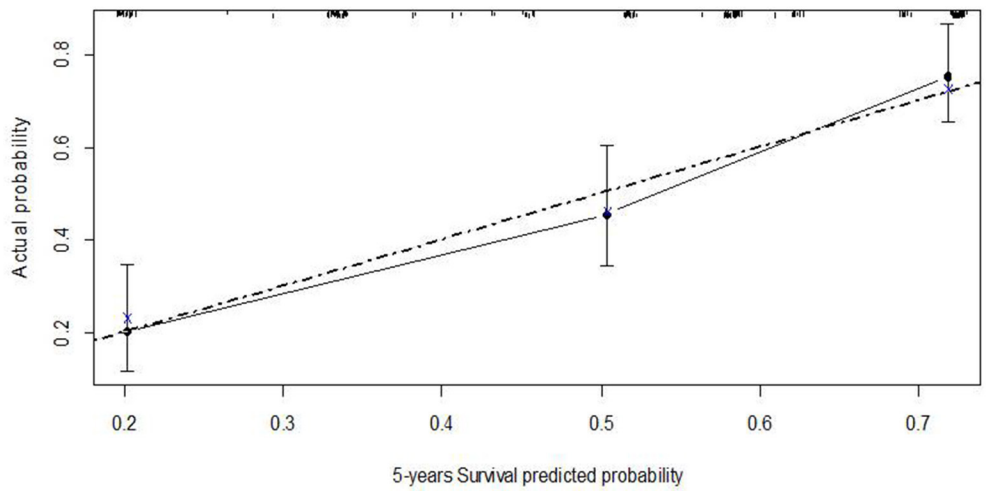

FIGURE 4 | Calibration plot of the Cox regression model of recurrence-free survival at five years–nomogram 1.

(16–18, 21–23). Tumor infiltration depth was associated with disease progression (pooled HR of 3.29, 95% CI: 2.39–4.51) (2–11, 11–22, 24, 25) and disease recurrence (pooled HR of 1.49, 95% CI: 1.11–2) (21, 22, 25). The meta-analysis concluded that both MM invasion and tumor infiltration depth subcategorization systems were strongly associated with both disease recurrence and progression after adjusting for the effects of established confounding factors (e.g., tumor grade, CIS, and multifocality).

In our study, RFS was significantly lower for $T1_c$ (compared with $T1_a$) and $T1_e$ (compared with $T1_m$). The comparison of the two substaging systems for their diagnostic performance in terms of RFS resulted in a slightly higher predictive power for $T1_{m-e}$.

The univariate Cox regression model documented that trigone or bladder neck location of the tumor, tumor diameter, number of tumors, substage $T1_e$, and squamous metaplasia were significantly associated with recurrence. In the multivariate model subcategory $T1_e$, the diameter, location, and number of tumors were confirmed as factors that significantly influenced recurrence after adjusting for the other variables in the model. All these factors are tumor-related and, consequently, not modifiable by the surgeon.

Concerning progression to muscle-invasive or metastatic disease, the log-rank test revealed a significant difference in PFS for $T1_c$ (compared with $T1_a$) and $T1_e$ (compared with $T1_m$). The comparison of the two substaging systems, again, yielded a higher prognostic accuracy for the $T1_{m-e}$.

The univariate and multivariate analyses confirmed that $T1_e$ and the diameter, location, and number of tumors were

significantly associated with PFS. The presence of the muscularis propria in the resection specimen (indicating deeper resection) shows a "protective" (pooled HR of 0.52, 95% CI:0.23–1.15), although not statistically significant, effect on tumor progression. Finally, concerning the efficacy of BCG instillations on progression, a protective effect was identified only for $T1_a$ ($p = 0.032$).

The nomograms that were tested documented a satisfactory agreement between the model predictions at 5 years and actual observations both for RFS and PFS.

A recent manuscript by Leivo et al. (26) identified five histopathologic features significantly associated with the progression of T1 BCa, irrespective of the presence of muscularis propria: any variant morphology, presence of LVI, necrosis, desmoplasia, and inflammation. Then, the authors used multiple different metrics to quantify T1 invasive tumor burden as either binary (single vs. multiple foci, focal vs. extensive invasion, above MM vs. into vs. below MM) or continuous [the aggregate linear length of invasive cancer (ALLICA), the % of specimen with invasive tumor, the calculated volume of invasive tumor, and the optical micrometer depth]. On the multivariate analysis, only three of these methods remained significant in predicting progression: ALLICA, focal vs. extensive invasion, and relationship to MM. The authors, however, underlined that the use of ALLICA eliminated the influence of the histopathologic features that have been related to progression. Concerning the focal vs. extensive method, the authors underlined the subjectiveness while the MM criteria could not be performed with certainty in 40% of patients.

As a general recommendation, although it is still debatable whether a linear measurement should be used instead of the MM invasion criteria, some strategies of pT1 substaging should still be attempted (13), as the literature clearly demonstrates the correlation between T1 substaging and clinical outcomes.

Finally, in our study, the BCG showed a significant impact on prolonging PFS only for $T1_a$ patients. This outcome is in line with the retrospective study of de Jong FC (27); the authors identified 264 patients with high-grade pT1 tumors and subdivided them as having extensive lamina propria invasion (73%) or microinvasion (27%) according to the substaging $T1_{m-e}$. With a median follow-up of 68 months, patients with $T1_e$ had a statistically significant difference in BCG failure (41 vs. 51%; $P = 0.002$). In the multivariable analysis, T1 sub classification was an independent predictor of high-grade RFS and PFS, indicating that patients bearing tumors with extensive invasion of the lamina propria are more likely to fail BCG therapy. This once again emphasizes the importance of quantifying the invasion of pT1 tumors as information that should be included in every pathology report.

The main limitations of our study are the retrospective design and the low numerosity of the patients combined with a relatively short follow-up, which may have hindered the final analyses and the effect of the BCG on both RFS and PFS for the various subcategories. Moreover, no *en bloc* resection of the BCa was adopted in the current series, which has been proven to improve the identification rate of MM and enhance the accurate identification of the T1 subcategory (28).

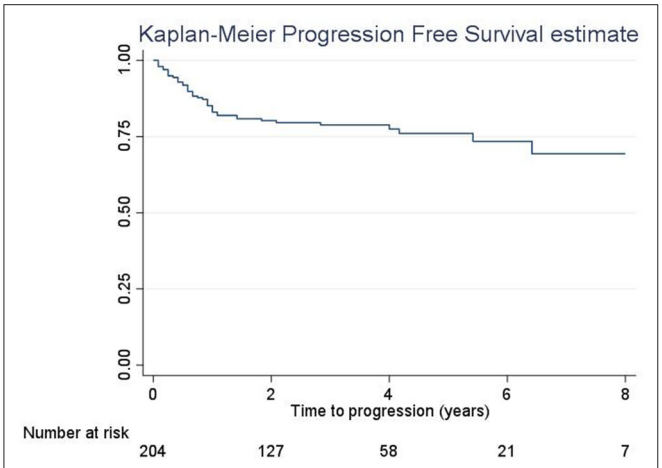

FIGURE 5 | Progression-free survival, overall.

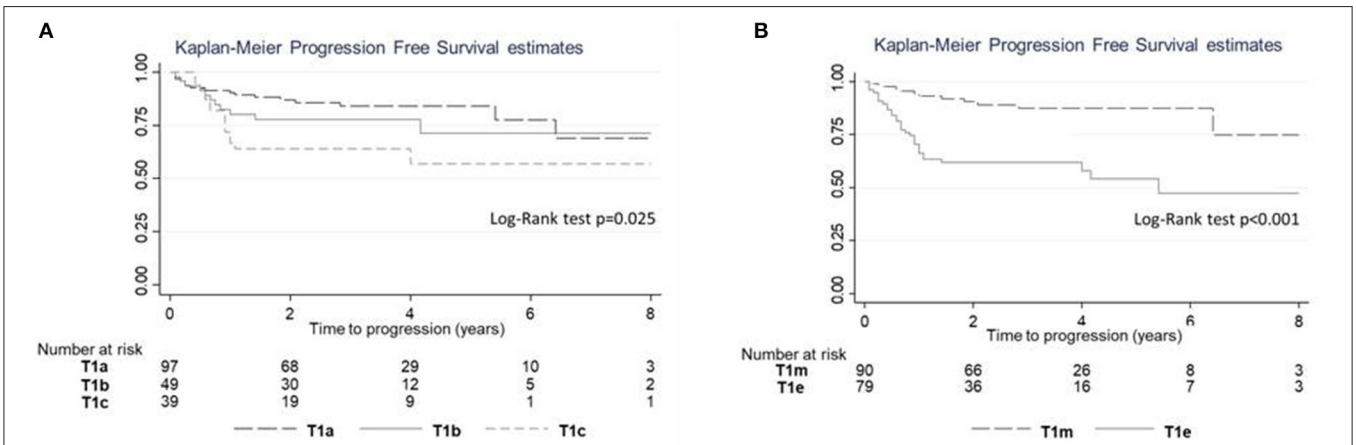

FIGURE 6 | Progression-free survival (PFS) estimates according to subcategory. **(A)** PFS for subcategories T1a–c. **(B)** PFS for subcategories T1m–e.

TABLE 7 | Uni and multivariable Cox regression model was performed to evaluate which independent variables influence the progression.

		Univariable				Multivariable*			
			95% CI				95% CI		
		HR	LL	UL	p	HR	LL	UL	p
Age, yrs		1	0.97	1.04	0.781				
Sex	M vs. F	0.67	0.3	1.51	0.338				
Smoking		1.01	0.56	1.83	0.974				
Neutrophil/lymphocyte, $n = 191$		0.89	0.78	1.01	**0.079**	Not in the model			
Trigone/bladder neck		2.66	1.47	4.81	**0.001**	2.04	1.08	3.84	0.028
Tumor diameter, $N = 203$									
	> 3 cm vs. < 3 cm	2.45	1.31	4.58	**0.005**	2.35	1.12	4.9	0.023
N of tumors									
	≥2 vs. 1	2.98	1.56	5.7	**0.001**	2.89	1.4	5.94	0.004
Substage T1a-c									
	T1b vs. T1a	1.48	0.68	3.18	0.32	Not in the model			
	T1c vs. T1a	2.54	1.25	5.15	**0.01**				
	Not evaluable vs. T1a	0.66	0.15	2.88	0.583				
Substage T1m-e									
	T1e *vs.* T1m	4.03	2.02	8.03	**<0.001**	3.5	1.56	7.85	0.002
	Not evaluable vs. T1m	0.47	0.1	2.1	0.319	0.69	0.14	3.26	0.636
Grade									
	G3 vs. G1	0.71	0.33	1.53	0.377				
CIS		5.24	1.85	14.85	**0.002**	Not in the model			
Muscolaris propria presence		0.53	0.27	1.05	**0.069**	0.52	0.23	1.15	0.107
Lymphovascular invasion, $n = 203$		2.71	1.36	5.38	**0.004**	Not in the model			
Tumor pattern	Solid	3.52	0.48	25.8	0.216				
Squamous metaplasia	Yes vs. no	0.58	0.08	4.2	0.588				
BCG instillations, $n = 199$	Yes	0.73	0.38	1.4	0.34				

Bold indicate the $p < 0.10$.

**N = 189.*

All variables with a $p < 0.1$ (Neutrophil/lymphocyte, Trigone/bladder neck, Tumor diameter, N of tumors, Substage T1m-e, CIS (carcinoma in situ), Lymphovascular invasion, Muscolaris propria presence) were considered for the multivariable analysis; stepwise selection method was applied to obtain the final model.

The suboptimal adoption of adjuvant BCG instillations is another limitation. In our study, the rate of patients submitted to BCG instillations was 70.4% and, as demonstrated in the univariate analysis (**Tables 6**, **7**), there was not any significant protective effect of the BCG both for progression (HR 0.73, 95% CI: 0.38–1.4, $p = 0.34$) and recurrence (HR 1.4, 95% CI: 0.87–2.25, $p = 0.162$). Although not specifically analyzed since it exceeded the purposes of this manuscript, the reasons for omitting the BCG may have been the higher age-related comorbidities, fear of severe side effects, or even the disbelief of the urologist with respect to the effectiveness of the BCG treatment, mainly for cases treated at the beginning of the current series (years 2009–2012). The suboptimal adoption of BCG instillations, however, has been commonly reported in the published literature. A recent population-based Scandinavian study (29) reports the total rate of T1 patients treated with BCG either early or delayed as 41%, with only 15% receiving BCG as early treatment (within 8 weeks after final diagnosis). In a Swedish T1 nationwide population-based study, the BCG rate was somewhat higher at ∼50% (30).

Low rates of re-TUR within 4 weeks and the absence of routine mapping during the repeated resection of the bladder are other limits of the study. With a strict definition of re-TUR as performed within 2–6 weeks from the first resection, only 20.2% of patients were submitted to re-resection. The reasons for the low uptake of re-TUR may be different; it may have been avoided, especially for low-grade disease at the initial TUR (the rate of G1 in this series was 12%) in elder or medically complex patients with severe comorbid conditions; other reasons for TUR avoidance may have been patient refusal or simply logistical reasons that prevented a re-TUR within the 8 week threshold. However, 167 (81.9%) of our pathologic examinations reported the presence of the muscularis propria in the resection specimen (with a consequent reduced risk of disease understaging), which is a significantly higher number with respect to other studies where the rate is <40% at the initial resection (31). In the latter study, the reported rate of RE-TUR was 22.4%. Low

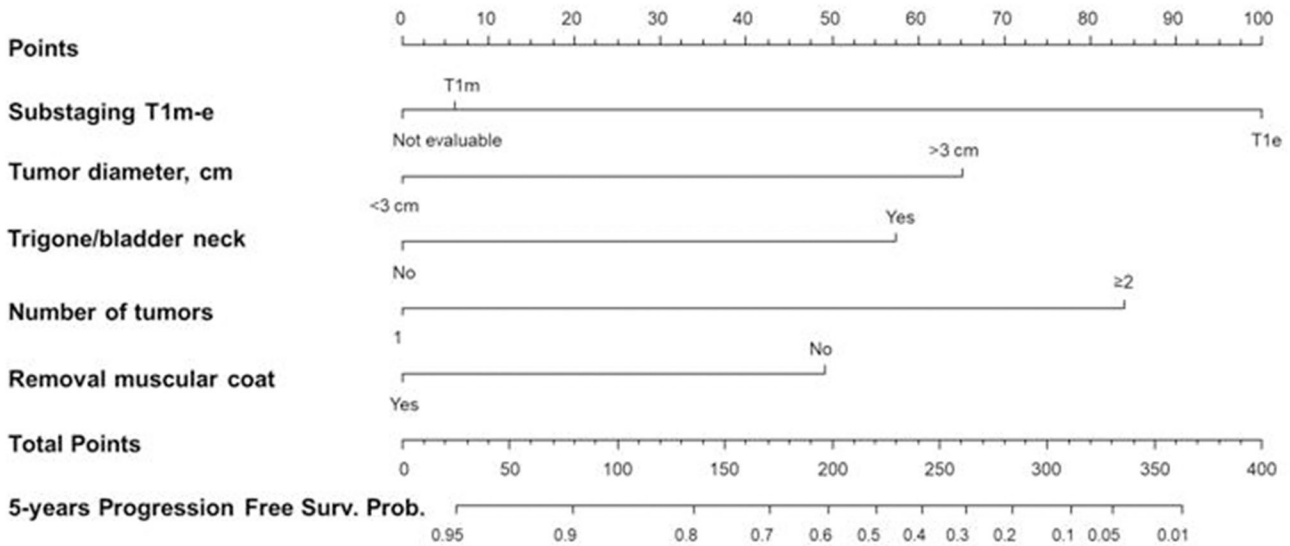

FIGURE 7 | Nomogram 2 for the calculation of the progression-free survival (PFS) at five years. On the basis of the estimated Cox regression model, a patient with T1e (100 points), >3 cm tumor diameter (65 points), trigone/bladder neck location of the tumor (57 points), number of tumors ≥2 (84 points), and with no presence of the muscle layer in the resected specimens (49 points) had a total score of 355 that corresponds to a low 5-year PFS probability ($p < 0.05$).

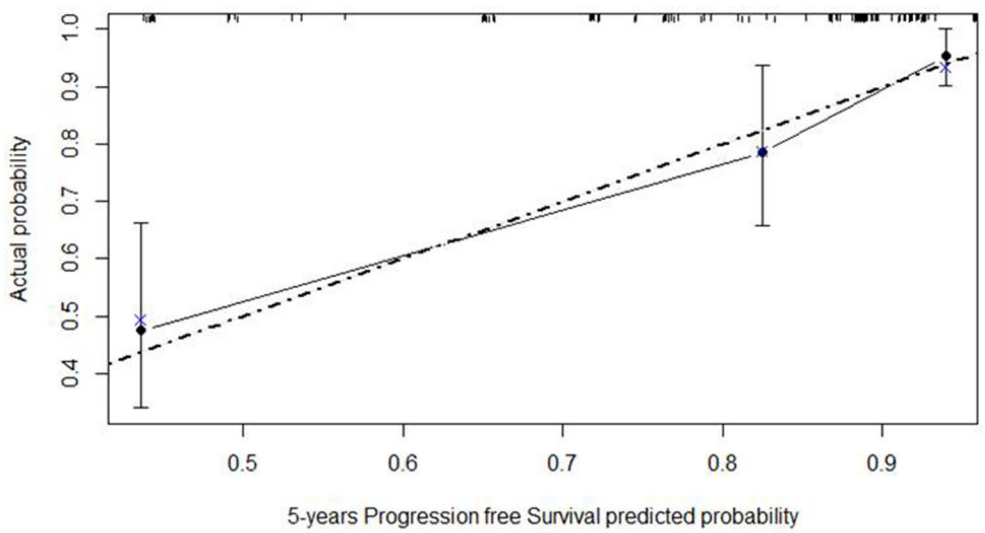

FIGURE 8 | Calibration plot of the Cox regression model of progression-free survival at five years of nomogram 2. The dashed gray line represents the "ideal" line of perfect correspondence between predicted and observed survival, the points represent the predicted "apparent" survival, and the vertical bars represent the 95% confidence intervals. The Xs represent bootstrap estimates corrected with n = 500 resamplings.

rates of re-TUR are commonly reported in published literature, reflecting the difficulties of adopting current guidelines in real-life practice (32).

The development of new risk-calculators that incorporate both the T1 subcategorization and the T1 molecular subtype as described by Robertson et al. (33), preferably on *en bloc* resection specimens, may overcome the limitations of the currently used models that show a poor discrimination for both disease recurrence and progression in NMIBC patients.

CONCLUSIONS

Both subcategorization systems ($T1_{a-c}$ and $T1_{m-e}$) are significantly associated with disease progression and recurrence for patients affected by T1 BCa, with $T1_{m-e}$ showing a slightly higher prognostic performance. Adequately designed prospective studies are necessary for the development of innovative risk calculators for the prediction of disease recurrence and progression and risk of BCG failure in T1 BCa; these models

should incorporate, besides the "traditional" prognostic factors, the T1 substaging.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Policlinico Tor Vergata ethics board, registration number 255.19. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

AA provided substantial contribution to the conception and design of the work as well as analysis and interpretation of data and drafting of the manuscript. AA, GCo, RT, GCa, AF, AM, RM, SD, SG, EF, VP, AC, and AP have revised the work critically for important intellectual content and provided final approval of the version to be published. They agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. GCo, RT, GCa, and AF provided substantial contributions to the acquisition and analysis of data for the work. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Surgery

REVIEW
published: 28 September 2021
doi: 10.3389/fsurg.2021.735868

Biomarkers in Bladder Cancer Surveillance

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Aim: This is a narrative review with an aim to summarise and describe urinary biomarkers in the surveillance of non-muscle-invasive bladder cancer (NMIBC). It provides a summary of FDA-approved protein biomarkers along with emerging ones which utilise genetic, epigenetic and exosomal markers. We discuss the current limitations of the available assays.

Background: Current guidelines advice a combination of cystoscopy, imaging,and urine cytology in diagnosis and surveillance. Although cytology has a high specificity, it is limited by low sensitivity particularly in low grade tumours. There are six FDA-approved urinary assays for diagnosis and surveillance of bladder cancer. They have shown to improve sensitivity and specificity to be used alongside cytology and cystoscopy but have a lower specificity in comparison to cytology and false positives often occur in benign conditions. Recent developments in laboratory techniques has allowed for use of markers which are RNA-, DNA-based as well as extracellular vesicles in the past decade.

Methods: Using the PubMed/Medline search engines as well as Google Scholar, we performed an online search using the terms "bladder cancer," "non-muscle invasive bladder cancer," and "urine biomarkers" with filter for articles in English published up to May 2021. Systematic reviews and original data of clinical trials or observational studies which contributed to the development of the biomarkers were collated.

Results: Biomarkers identified were divided into FDA-approved molecular biomarkers, protein biomarkers and gene-related biomarker with a table summarising the findings of each marker with the most relevant studies. The studies conducted were mainly retrospective. Due to the early stages of development, only a few prospective studies have been done for more recently developed biomarkers and limited meta-analyses are available.Therefore a detailed evaluation of these markers are still required to decide on their clinical use.

Conclusion: Advancements of analytical methods in BC has driven the research towards non-invasive liquid-based biomarkers in adjunct to urine cytology. Further large prospective studies are required to determine its feasibility in a clinical setting as they are not effective when used in isolation as they have their limitation. With the ongoing pandemic, other than reduction in costs and increased accuracy, the need for biomarkers to cope with delay in cystoscopies in diagnosis and surveillance is crucial. Thus clinical trials with direct comparison is required to improve patient care.

Keywords: biomarker, bladder cancer, surveillance, non-muscular invasive bladder cancer, cancer screening

OPEN ACCESS

Edited by:
Daniele Castellani, Polytechnic University of Le Marche, Italy

Reviewed by:
Bryan Kwun-Chung Cheng, United Christian Hospital, Hong Kong SAR, China
Peter Ka-Fung Chiu, The Chinese University of Hong Kong, China
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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *03 July 2021*
Accepted: *25 August 2021*
Published: *28 September 2021*

Citation:
Sugeeta SS, Sharma A, Ng K, Nayak A and Vasdev N (2021) Biomarkers in Bladder Cancer Surveillance. Front. Surg. 8:735868. doi: 10.3389/fsurg.2021.735868

INTRODUCTION

Bladder cancer accounts for 90–95% of urothelial cancers. It is the eight most common cancer in women and fourth most common cancer in men (1). Most cases present with non-muscle invasive bladder cancer (NMIBC) and at early stages this carries a favourable prognosis. However, NMIBC accounts for 75% of cases and has a high recurrence rate of 80% in high-risk lesions and up to 50% in low risk. The 5 year survival rate is 94% if detected early and therefore early detection is imperative as intervention drastically influences overall survival (2).

Currently, bladder cystoscopy in combination with imaging of the upper urinary tract along with voided urine cytology as part of surveillance. NICE guidelines recommend cystoscopy to be done every 3 months for the first 2 years then every 6 months for the next 2 years then once a year thereafter. Cystoscopy is associated with complications such as a urinary tract infection, haematuria, pain. The utilisation of both imaging and cystoscopy is not effective in detecting smaller lesions. Urine cytology remains the most widely used non-invasive method for both diagnosis and surveillance of BC. Studies have shown a high specificity of 86% but this is limited by its low sensitivity of 48% as there is subjective nature when grading urothelial carcinoma on urine samples resulting in poor inter-observer variability (3). Although routinely used as the standard of truth for assessment of diagnostic accuracy, it is well-recognised that traditional cystoscopy with use of white light can lead to missing lesions that are present but not visible. New technologies exist to improve tumour visualisation. A recent study compared the use of blue light flexible cystoscopy with hexaminolevulinate (HAL, Hexvix®, Photocure ASA) with white light flexible cystoscopy for the detection of bladder cancer during surveillance, finding that 20.6 % (95% CI 11.5–32.7, $p < 0.0001$) of patients with recurrent cancer was seen only with blue light (4). The fact that a significant proportion of recurrences are missed under white light cystoscopy should be taken into consideration when assessing the sensitivity of new markers.

Urinary biomarkers play an important role in the future of precision medicine given the limitations of the current modalities being used given the specificity and sensitivity and need for invasive procedures to allow for surveillance. There is also a significant impact due to costs involved to healthcare services given the frequency and reliance on cystoscopy at present. This has led to the development of several non-invasive biomarkers which are now FDA approved. This is now particularly relevant with regard to low and intermediate risk patients who have had cystoscopies deferred with the ongoing pandemic. UroFollow is a multi-centre prospective trial exploring follow up using urine biomarkers in comparison to standard of care to explore if non-invasive methods are sufficient or patients with low grade or pTa G1–G2 BC (5). In addition to diagnostic accuracy, biomarkers need to be reproducible tests, affordable and easily implementable. This review's aim is to summarise biomarkers which have been identified for use in BC surveillance which are FDA-approved, commercially available and potential biomarkers in development.

FDA-APPROVED MOLECULAR BIOMARKERS

The United States Food and Drug Administration (FDA) have currently approved 6 urinary assays to use alongside cystoscopy for diagnosis and surveillance. These include BTA stat (Polymedco), BTA TRAK (Polymedco), NMP22 enzyme linked immune-sorbent assay (ELISA) (Matritech), NMP22 BladderChek Test (Alere), uCyt (Scimedx) and UroVysion (Abbott Molecular).

NMP22

Nuclear matrix proteins are non chromatin structures which play several roles from DNA replication to gene expression and contributes to the infrastructure of the cell nucleus. During replication in healthy cells, NMP22 regulates the distribution of chromatin to daughter cells and this is normally at low levels. In urothelial tumours, levels of NMPs are high due to cell turnover from tumour apoptosis. NMP22 is one of them and it is the most investigated as an assay in both diagnosis and recurrence of bladder cancer.

Two modes of detection were FDA approved for both diagnosis and surveillance. NMP22 were initially detected with quantitative ELISA in a laboratory where cut-off values were utilised. The second was a qualitative point-of-care test, the NMP22 BladderChek where monoclonal antibodies are used to detect raised NMP22 levels in BC.

In 2015, Chou et al. had done a meta-analysis identifying qualitative NMP22 which has a sensitivity of 69% and specificity of 77% and qualitative NMP22 has 83% in specificity and 70% in sensitivity (6). A meta-analysis by Wang et al. in 2017 showed a pooled sensitivity from of 56% and specificity of 88% for bladder cancer detection from 19 studies (7). However the sensitivity was low when tumour stage and grade were considered with sensitivity increasing steadily with stage of 13.68, 29.49, and 74.03% for Ta to T1 and >T2, respectively. It was also found to have a better diagnostic performance in the Asian population. NMP22 measures the cell turnover that occurs with surface shedding from bladder tumour. This process occurs in benign conditions such as inflammation, infection, bladders stones and haematuria thus resulting in false-positive results.

Bladder Tumour Antigen (BTA) Assays

BTA tests detect human complement factor-H related protein in the urine which is produced by our bodies to protect cells from complement activation. It has an almost identical structure to the complement factor-H related protein produced by bladder cancer cells. There are two forms of BTA assays: (1) BTA Stat test: a "point-of-care (POC)" immunochromatographic assay which utilises five drops of urine to deliver a result within 5 min, and (2) BTA-TRAK test: standard quantitative ELISA measurement of the antigen. The FDA have approved them both for surveillance in BC in conjunction with cystoscopy only.

A meta-analysis conducted reviewing 13 studies identified specificity and sensitivity of BTA stat test to be 67 and 75%, respectively (8). Although BTA stat had shown higher sensitivity that urine cytology, the latter had better specificity. Chou et al.

reviewed 22 studies identifying the sensitivity of BTA STAT was 64 % and specificity was 77%. For BTA-TRAK, four studies were evaluated and had similar results with a sensitivity of 65% and specificity was 74% (6). Similarly to other biomarkers, sensitivity had a positive correlation with increasing tumour grade of the BC.

Overall, the sensitivity and specificity of BTA Stat test ranges from 56 to 83% and 64 to 86% and with specificity up to 93% in individuals with benign conditions (9–12), and with BTA TRAK this ranges from 62 to 76% and 51 to 98% (13). Glas et al. carried out a meta-analysis and results of the bivariate analysis showed a sensitivity and specificity of cytology, BTA-Stat and BTA TRAK to be the following 55 and 94%; 70, 75, and 66%, and 65%, respectively (14). Given their lower specificities and similar issue of false positive results in benign conditions such as previous intravesical therapy, kidney stones, infection and presence of ureteric stents or nephrostomy tubes, theses tests are unable to replace cytology and can only be used concurrently as part of surveillance (9, 12, 15, 16).

UroVysion

UroVysion is a molecular test using multicolour fluorescence *in situ* hybridisation (FISH) assay to detect aneuploidy of chromosomes 3, 7, and 17 and loss of the p16 gene at the 9p21 locus which are genetic abnormalities seen in BC. A sample must have a minimum of 25 cells to be analysed and a positive test is defined by one of the following: (1) Four or more morphologically abnormal cells have polysomy of two or more chromosomes (3, 7, or 17) (2) $\geq$10 cells with gain of a single chromosome (3) homozygous deletion of 9p21 in 12 cells (16).

Pooled results from a meta-analysis of 13 studies showed a specificity of 83% and sensitivity of 72% in comparison to urine cytology with 96 and 42%, respectively (17). UroVysion showed the sensitivity and specificity of 75.6 and 84.8%, respectively for high grade UCC, 40.8 and 87.8%, respectively for lowgrade UCC (18). Thus it faced a similar challenge to biomarkers in detection of low grade or low stage tumours (19). However the advantage of this assay is the absence of benign conditions such as cystitis, inflammation or haematuria affecting results.

In surveillance, Yoder et al. identified over a period of 29 months that 65% of the cases with positive UroVysion but no visible lesions developed recurrence on follow-up but this was not the case for Dimashkieh et al. as where 46% (158 of 343) of patients who had positive UroVysion tests did not develop UCC during up to the 3 year follow-up (18, 20). Therefore there is variability in its clinical utility. There is also evidence to show its potential in assessing response to intravesical BCG therapy for NMIBC (21).

Immunocyt/Ucyt + Test

The Immunocyt/Ucyt+ test uses three fluorescent monoclonal antibodies (M344, LDQ10, and 19A211) which detect carcinoembryonic antigen and sulphated mucin glycoproteins on exfoliated urothelial cells in voided urine. Compoj et al. evaluated 7,244 cases and identified an overall sensitivity of 34.5% for cytology and 68.1% for uCyt+/ImmunoCyt and 97.9% for cytology, 72.3% for uCyt+/ImmunoCyt (22). There was a positive correlation with higher grade and specificity along with sensitivity as observed with other biomarkers. A meta-analysis identified a sensitivity of ImmunoCyt to be 75% and specificity was 78% and in comparison to NMP22, BTA and FISH, it had the highest pooled sensitivity (6). A split study comparing UroVysion, ImmunoCyt and cytology supported this with Immunocyst being more sensitive in detecting low grade tumours (23). Further meta-analysis also supported previous evidence to support the use of Immunocyt in combination with cytology in surveillance to reduce the frequency of follow up required in low risk cancers (24).

However, this test involves advanced technical expertise as a minimum of 500 cells need to be analysed for fluorescence to provide accurate results and thus there is interobserver variability and need for high cellularity specimens. It can be affected by benign conditions such as haematuria albeit not as easily as other biomarkers above (25).

PROMISING PROTEIN BIOMARKERS

Table 1 summarises potential protein biomarkers which can be used in BC surveillance highlighting meta-analysis and most relevant study in the table.

The UK National Health Service (NHS) approved the usage of ADxBladder in BC detection. Three prospective studies have been done with only one in the surveillance setting (43). They reported an overall sensitivity ranging between 45–73%, specificity between 70–73%, and NPV between 74–100% and were superior to cytology (47). Given the turnaround time of 2h, it being relatively unaffected by benign conditions such as inflammation or haematuria and consisting of a single biomarker identifiable with ELISA which is readily available in labs and costing only £0.37 per person, this made ADxBladder a viable option (48). However, in comparison to other biomarkers identified, it has a low sensitivity and specificity as displayed in **Table 1** and poor performance in detection of low grade tumours.

Of the new protein biomarkers that were introduced in recent years, URO17 test utilising Keratin 17 (K17) has shown especially promising results. Babu et al. had identified high sensitivity and specificity of URO17 of 100% using urine samples in a retrospective study (44). Interestingly, URO17 is able to detect both low and high grade cancers in patients presenting with haematuria, a previously excluded cohort, thus proving its benefit of use in a surveillance setting as well (45). There is a specificity of 96 and 92.7% in recurrent and newly diagnosed patients, respectively (44, 45). Given these outcomes and its easy adaptation to current equipment used, a larger prospective study in a surveillance setting would be beneficial in developing this into a promising protein biomarker test for non-invasive surveillance of NMIBC (45, 46).

GENE-RELATED BIOMARKERS

Genetic alterations has been explored as another avenue for detecting bladder cancer in surveillance. To discuss this further we will divide these into the following groups:

TABLE 1 | Additional protein biomarkers.

Biomarker	Description	Method	N	C	SS	SP	Comment	Ref
UBC	Detects the presence of fragments of cytokeratin 8 and 18 in urine	ELISA or immunoradiometric assay	753	1072	64.4	80.3	UBC values higher in high-grade tumours and able to distinguish from low-grade. Higher specificity in combination with cytology or survivin assay	(26–29)
CYFRA21-1	Quantifies soluble fragments of cytokeratin 19	ELISA	1262	1233	82.0	80.0	Significantly higher levels in patients with metastatic disease vs. locally invasive unable to differentiate between histological grades	(30, 31)
BLCA-4	Measures protein components of the nuclear matrix which are present in the urothelium of BC patients	ELISA	1119 total participants		93.0	97	Meta-analysis mainly retrospective studies showing potential to detect early tumours. No positive correlation between tumour stage and levels measured.	(32, 33)
CellDetect	Composed of a unique plant extract which interacts with malignant cells due to their increased metabolic activity	Immunostain	84	110	84.0	70.0	Two studies have shown higher sensitivity in low grade tumours in comparison to urine cytology (82% vs 59%) and similar specificity (86 vs 94%) It was also found not be affected by haematuria.	(34, 35)
Hyaluronic acid	HA is a glycosoaminoglycan and HAse is endoglucosidase involved in tumour metastases and breakdown of HA into fragments for angiogenesis	ELISA and RT-qPCR	918 participants		90.8	82.5	In comparison to BTA stat, UBC and cytology in two studies shown superior SS and SP. SS and SP not affected by tumour grade but levels are not indicative of tumour grade. More studies required to evaluate this promising marker.	(36–38)
sFas	Anti-apoptotic protein released by BC cells to protect from anti-tumour activity	ELISA	128	88	51.2	85.9	Lower sensitivity. Higher levels associated with higher risk of recurrence.	(39–41)
Survivin	Overexpression in BC as a protein which inhibits apoptosis pathways	Bio-dot test	50	44	82	90	Limited data available in follow up setting or in comparison to other biomarkers	(42)
MCM5 - ADXBladder	Detects MCM5 shed by replicating BC cells	ELISA	503 patients		51.9	66.4	Findings in prospective study in comparison to UC with SS 16.9% and SP 98%. Low sensitivity for low grade tumours. 99% NPV for high risk NMIBC	(43)
URO17	Detects oncoprotein Keratin 17 involved in the replication of cancer cells	Immunocyto-chemistry	81	98	97	AUC: 90	Consistently high sensitivity and specificity from 3 independent studies. Good potential as simple incorporation to existing equipment	(44–46)

N, tumour samples; C, control; SS, sensitivity; SP, specificity; UBC, Urinary bladder cancer; BLCA-4, Human Bladder Cancer-associated Nuclear Matrix Protein4; HA, hyaluronic acid; HAse, hyaluronidase; sFas, soluble Fas; RT-qPCR, Real-Time Quantitative Reverse Transcription; BC, bladder cancer; UC, Urine cytology; NPV, negative predictive value; NMIBC, Non-muscular invasive bladder cancer; AUC, Area under curve.

DNA methylation markers, histone tail modifications, miRNA biomarkers, microsatellite analysis and multi-gene panels.

Table 2 summarises detection of genetic alterations to utilise as biomarkers listing the most relevant study accounting for those with the largest patient groups and most representative of the target group.

DNA Methylation Markers

Epigenetic alterations are part of the carcinogenesis. DNA hypermethylation of the CpG islands play a role in the promoter regions of tumour-suppressor genes. This mediates silencing of the affiliated gene which is a known phenomenon in BC. The hyper or hypomethylation of these genes can be detected in tumour cells that are shed into urine and aid diagnosis of BC. This has been reviewed in both primary and recurrent tumours. Bosschieter et al. evaluated 42 studies and identified 8 with high sensitivity and specificity with varying methodologies and heterogenous patient groups (66). Studies with promising results, had no independent validation data and Costa et al. with 94% specificity and 90% sensitivity did not report on tumour grade or stage (67). This is relevant as similarly to other reported biomarkers, results will vary with disease spectrum. As shown in **Table 2** section DNA Methylation Markers studies in recurrence are listed but these are mainly small retrospective case-control studies (50). Beuker at al, as shown in **Table 2**, used a combination of this technique along with DNA mutation analysis with FGFR3 and TERT mutation analysis to improve detection rates showed similar results as increased sensitivity in high grade tumour cells likely due to increased shedding of BC cells (49). There is an insufficient amount of data due to variability in methodology, patient groups and gene panel selection. Studies have used it in combination with DNA mutation analysis.

Histone Tail Modifications

Other than epigenetic changes described above, another manifestation of this is histone lysine methylation (HxKy). Histone modifications help regulate numerous cell mechanisms such as chromosome condensation, DNA repair and transcription. The site and degree of histone methylation determines the transcriptional activity. H3K9, as mentioned in **Table 2** section Histone Tail Modifications are associated with repressed transcription. Other potential histone modications identified are H3K4 and H3K20 methylation which were decreased in BC compared to normal patients and global H4K20me3 levels were predictive for bladder cancer-specific survival (68).

miRNA Biomarkers

Micro RNAs (miRNA) are short noncoding RNAs that regulate process post-transcriptionally and dysregulation leads to carcinogenesis. The aberrant expression of miRNA has led to its potential use as a biomarker. It can present in bodily fluids as they are protected by RNAse degradation because they are excreted as membrane-protected free circulating miRNAs or in extracellular vesicles (EVs) such as exosomes (69). Initially identification was done using qtPCR but now rapid profiling is done using microassays and miRNA sequencing (56).

Studies listed in **Table 2** section miRNA Biomarkers are those with sensitivities of more than 80%. Multiple miRNA diagnostic assays had better sensitivity than single miRNA assays. Chen et al. carried out a meta-analysis 30 studies with 1019 BC patients and 690 controls identifying a pool sensitivity and specificity of 80 and 74%, respectively (70). The AUC for NMIBC was 0.84 and 0.76 for MIBC suggesting higher diagnostic ability in NMIBC patients. Another meta-analysis by Shi et al. evaluated 1,556 cases and 1,347 controls from 31 studies with a pooled specificity and sensitivity of 72 and 76%, respectively (71).

Most studies compared a heterogenous group of BC patients with controls. Study which explored the recurrence setting in both NMIBC and MIBC using miR-145 and miR-200a. This identified a sensitivity of 78% and specificity of 61% in NMIBC patients where lower levels of miR-145 associated with higher grade and lower levels of miR-200a independently predicted recurrence (72). Prospective trials in BC surveillance are required to validate the clinical applicability of this biomarker.

Multi-Gene Panels

Several assays detecting mRNA biomarkers have been conducted. **Table 2** section Multi-Gene Panels summarises them. CxBladder has been extensively studied and has variations which include: (1) Cxbladder® *Detect* to detect bladder cancer in hematuria patients with a sensitivity of 82% and specificity of 85% (62). (2) Cxbladder® *Triage* which is used in hematuria patients to rule out BC with a sensitivity of 95% and negative predictive value of 97% (73). (3) Cxbladder® *Monitor* as a complement to surveillance. It has been compared with urine cytology, NMP22 BladderChek and NMP22 ELISA with a superior SS and SP of 91/96 vs. 22/87%, 11/87 and 26/86 % (74). Koya et al. implemented CxBladder Monitor (CxBM) into local guidelines whereby low risk patients had alternate annual CxBM and cystoscopy therearfter (75). They found that 77.8% of patients were safely managed by only one cystoscopy every 2 years, reducing the total number of annual cystoscopies by 39%. This was reflected in a real world data analysis identifying this advantage of CxBM in clinical practice to have driven its increased utility (76).

Other Possible Gene-Related Biomarkers

Microsatellite analysis (MSA) through PCR targets highly pleomorphic short tandem repeats (STR) which occur in cancer cells with loss of heterozygosity (LOH) causing microsatellite instability. This occurs because of epigenetic silencing or inactivation of the mismatch repair gene which play an integral part in the proliferation of cancer cells. The most common LOH is in chromosome nine but this is also seen to occur in chromosome 4, 8, 11, and 17p (77–80). In comparison to urine cytology, sensitivity was 97 vs. 79% with 95–100% in low grade tumours in a small study of 34 cancer patients with 21 cancer-free subjects (81). A prospective study of 228 patients undergoing BC surveillance had a specificity and sensitivity of 58 and 74%, respectively (82). A further prospective study of 91 patients evaluating MSA in combination with cytology had a sensitivity of 72% in G1–2 and 96% in G3. They found using LOH analysis to improve specificity and all recurrence cases were identified (83).

TABLE 2 | Gene-related biomarkers.

	Biomarker	Description	M	N	C	SS	SP	Comment	Ref
4.1	*FGFR3*, *TERT* and *OTX1*	Combination of DNA methylation levels and DNA mutation analysis	SNaPshot®	977 pts 2496 sp		LG: 57% HG: 72%	LG: 59% HG 59%	Large prospective study identified lower sensitivity in follow up HG and LG patients in comparison to primary LG and HG. Increased sensitivity with HG tumours.	(49)
	HS3ST2, SEPTIN9, and SLIT2/FGFR3	Detects 4 hotspot mutations in FGFR3 in combination with DNA methylation levels	qMS-PCR	157		63	58	Combination with methylation shows sensitivity of 94% in low grade and 100% in high grade to 100% in recurrence	(50)
	SOX-1, IRAK3, and Li-MET [	Detecting changes in DNA methylation in BC cells shed in urine		90		86	89	Tumour recurrence predicted in 80% of patients, superior to cytology (35%) and cystoscopy (15%)	(51)
	APC _ a, TERT _ a, TERT _ b, and EDNRB		MS-MLPA	49	60	72	55	All HG recurrent tumours including CiS were detected but pTa G1-2 were missed	(52)
4.2	Histone tail modifications (HTF) H3K9 and H3K27	HTFs help regulate cell processes which are fundamental to DNA repair. Low levels have been associated with BC	Immuno-histochemical analysis	113	61	N/A		H3K9 and H3K27 levels correlate with invasiveness of BC along with grade and pT stage of NMIBC, Expression of H3K27me3 predictor for cancer-specific survival post-cystectomy.	(53)
4.3	Has-let-7c, miR-135a, miR-135b, miR-148a, miR-204, miR-345	Analysis of 364 different miRs was analysed in 16 urine samples to identify a 6 gene signature	miRNA assay	130‘	112	AUC 88.3%		AUC of 88.0%, 92.9% and 91.0%, for LGNMIBC, HGNIMBC and MIBC respectively. Validation sets were done. Newly diagnosed patients only, limiting clinical applicability in surveillance.	(54)
	6 step: **miR-187, miR-18a, miR-25,** *miR142-3p, miR-140-5p, miR-204* 2 step: miR-92a, miR-125b	In bold, overexpressed in BC and in italics, they were under expressed.	miRNA assay	27	10	85 85	87 74	2 step model predicts progression and cancer specific survival in NMIBC patients. However, only a small case control study	(55)
	miR16, miR200c, miR205, miR21, miR221 and miR34a	Panel of 12 miRNAs profiled and identified set of 6. Validation study included.	RTq-PCR	81	21	88	48	AUC 0.85 in predicting recurrence in surveillance setting. Better performance in larger tumours and higher T-stage	(56)
	miR-21, miR-15a, miR-200c miR-93, miR-191, and miR-940, Has-let-7b	Seven miRNA identified to have significantly higher levels in the cancer group	RTq-PCR	85	45	88	78	No validation study. Highest sensitivity in T1G3 and >T2 patients.	(57)
	25 target diagnostic miRNA signature	A panel of 46 miRNAs monitored in an independent cohort of 121 subjects identifying 25-target panel	RTq-PCR	60	61	87	100	No validation cohort. Further prospective studies required.	(58)

(Continued)

TABLE 2 | Continued

	Biomarker	Description	M	N	C	SS	SP	Comment	Ref
4.4	Xpert Bladder	Detects expression levels of 5 mRNA expression of genes (CRH, IGF2, UPK1B, ANXA10, and ABL1)	LDA	239	-	74	80	Prospective study. Higher SN and NPV compared with cytology and UroVysion with NPV of 98% in HG disease	(59)
	AssureMDX	Mutation analysis in FGFR3, TERT, and HRAS genes and methylation analysis in OTX1, ONECUT2, and TWIST1 genes	SNaPshot® + MS-PCR	74	80	97	83	Follow up validation study showed SS of 93 and SP of 86. AUC higher in >Ta compared to Ta and om HG tumours	(60, 61)
	CxBladder	IGFBP5, HOXA13, MDK, and CDK1 are associated with carcinogenesis and the 5th biomarker (CXCR2) is a marker of inflammation reducing false positive results	Rt-qPCR	66	417	82	85	97% SS in HG tumours and 69% in Ta. Greater SS in comparison to urine cytology and NMP-22	(62)
	EpiCheck	Blinded, single-arm, prospective multicenter study using Epicheck (15 proprietary DNA methylated genes) in NMIBC surveillance	Rt-qPCR. EpiScore	353	-	68	88	SS 100%, 40%, 89% in Cis, LG and HG respectively.	(63)
	Uromonitor	FGFR3 hotspot and TERT promoter mutations	Rt-qPCR	122	-	73	73	Addition of KRAS mutation to UroMonitor V2 kit, increased SS to 100% and specificity to 83.3%	(64)
	UroSEEK	Mutations in 11 genes or presence of abnormal number of chromosomes	NGS	496	-	74	72	UroSEEK positivity preceded tumour recurrence diagnosis by 4 months in average. However, this is a retrospective study and prospective studies needed.	(65)

M, Method; N, number of malignant samples or cancer patients; C, control; SS, sensitivity; SP, specificity; Ref, reference; LG, low grade; HG, high grade; Cis, Carcinoma in situ; qMS-PCR, quantitative methylation specific-polymerase chain reaction; Rtq-PCR, Quantitative reverse transcription PCR; AUC, area under curve; NGS, next-generation sequencing assay; LDA, linear discriminate analysis.

Overall, this test has good sensitivity but difficult to incorporate into present laboratories due to its complexity. Larger prospective studies which include a validation cohort to assess feasibility is required.

EXTRACELLULAR VESICLES AND EXOSOMES

Exosomes are vesicles secreted by cells which mediate extracellular communication by transmitting proteins, lipids, miRNA, mRNA and long non-coding RNAs (lncRNA). Extracellular vesicles in BC cells carry a vast number of proteins which is utilised in angiogenesis and cell migration thus aiding further tumour progression which can be utilised as markers but possible novel therapeutic targets (84). Different analytical methods have identified various proteins and miRNAs in this rich extracellular environment. A small study isolated EV using microchip ELISA and found highly elevated EV levels in BC patients compared to controls (85). Profiling of proteomes in exosomes identified a correlation with tumour grade and the ability to predict recurrences with 1-antitrypsin and histone H2B1 exosome proteins (86). In another study, urinary EVs were isolated and deregulated miRNAs were identified as potential biomarkers, in particular, miR-375 for high-grade bladder cancer while miR-146a for low-grade patients (87).

EVs are promising as a source of biomarkers given the diverse cargoes EVs carry. However, there are several limitations such as isolation techniques and testing not being standardised and therefore it is difficult to compare results between groups given lack of reproducibility. Studies conducted are heterogenous and small without any validation sets (88). Optimisation of testing could lead to better EV studies allowing for real EV biomarker development in a clinical setting and could further inform us further on tumour biology.

CONCLUSION

This paper has highlighted the various biomarkers in urothelial cancer and their significance in early diagnosis of bladder cancer. Whilst it's important to have biomarkers in NMIBC, differences in sensitivity and specificity limits their use in the community. These biomarkers have a significant role in future diagnosis of bladder cancer, and future studies will guide clinicians in using the most appropriate marker for screening. The advancement in analytical methods in BC has driven the research towards non-invasive liquid-based biomarkers in adjunct to urine cytology. This paper provides evidence that a second modality of screening tool may be beneficial to use in the diagnostic algorithm for bladder cancer. Studies identifying its feasibility in a clinical enviroment is important as they have limitations when used in isolation. Given this is a narrative review, further evaluation of these promising markers is required in more depth in terms of a meta-analysis along with the development of prospective studies in a surveillance setting. Meta-analyses on the newer markers have not been conducted as there is variability in patient cohorts utilised and more studies need to be conducted to obtain sufficient data. In addition to this, majority of studies are in a restrospective setting and prospective studies need to be developed to be able to further evaluate their clinical feasibility.

The ongoing pandemic has further accentuated the increasing need and relevance for biomarkers to cope with delay in cystoscopies in both diagnosis and surveillance. The use of more sensitive methods to detect true tumour recurrences could also play a role in assessment of the diagnostic accuracy of these markers, potentially reducing the number of cystoscopies for test-negative cases and introducing methods like blue light cystoscopy for test-positive cases. Further large, prospective clinical trials incorporating these biomarkers and usage of newer analytical methods in screening for high-risk patients and also in disease recurrence in will allow for its use in a clinical setting.

AUTHOR CONTRIBUTIONS

SS collated and summarised studies to write up majority of this review. KN and AN were involved in editing. AS and NV contributed to the introduction and conclusion. All authors contributed to the article and approved the submitted version.

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frontiers in Surgery

ORIGINAL RESEARCH
published: 08 November 2021
doi: 10.3389/fsurg.2021.759487

Thulium Laser Resection of Bladder Tumors vs. Conventional Transurethral Resection of Bladder Tumors for Intermediate and High Risk Non-Muscle-Invasive Bladder Cancer Followed by Intravesical BCG Immunotherapy

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Background: Thulium laser resection of bladder tumors (TmLRBT) is recently considered as a common treatment option for non-muscle-invasive bladder cancers (NMIBC), but whether it is superior to Transurethral resection of bladder tumors (TURBT) are still undetermined.

Materials and Methods: We retrospectively screened our institution database to identify patients who were treated by conventional TURBT or TmLRBT for NMIBC and followed by intravesical bacillus Calmette-Guérin (BCG) immunotherapy. The preoperative characteristics, perioperative outcomes, and recurrence-free survival were compared to assess the safety and efficacy of the two procedures.

Results: Eventually, 90 patients who underwent TmLRBT ($n = 37$) or TURBT ($n = 53$) followed by intravesical BCG immunotherapy were included. Two groups were similar in baseline characteristics except for the smaller tumor size of the TmLRBT group(1.7 cm vs. 2.2 cm; $P = 0.036$). Obturator nerve reflex occurred in eight patients in the TURBT group and 3 of them suffered from bladder perforation while none happened in the TmLRBT group. The TmLRBT also had a shorter irrigation duration. In the multivariate Cox regression, the TmLRBT was related to less recurrence risk (HR: 0.268; 95% CI, 0.095–0.759; $P = 0.013$).

Conclusion: Our results suggested that TmLRBT is safer than conventional TURBT with fewer perioperative complications, and it offers better cancer control, therefore might be a superior option for NMIBC patients with intermediate and high recurrence risk.

Keywords: thulium laser, en bloc resection of bladder tumor, non-muscle-invasive bladder cancer, bacillus Calmette-Guérin vaccine, transurethral resection of bladder tumors

OPEN ACCESS

Edited by:
Jeremy Teoh, The Chinese University of Hong Kong, Hong Kong SAR, China

Reviewed by:
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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *16 August 2021*
Accepted: *18 October 2021*
Published: *08 November 2021*

Citation:
Liu Z, Long G, Zhang Y, Sun G, Ouyang W, Wang S, Xu H, Wang Z, Guan W, Yu X, Hu Z, Chen Z, Wang S and Li H (2021) Thulium Laser Resection of Bladder Tumors vs. Conventional Transurethral Resection of Bladder Tumors for Intermediate and High Risk Non-Muscle-Invasive Bladder Cancer Followed by Intravesical BCG Immunotherapy. Front. Surg. 8:759487. doi: 10.3389/fsurg.2021.759487

INTRODUCTION

Bladder cancer ranks second common urological malignancy worldwide (1). It represents a spectrum of diseases, from non-muscle-invasive bladder cancer (NMIBC), which is defined as the tumor confined to the bladder mucosa or submucosa, to invasiveand advanced diseases that demand aggressive treatment. Approximately 75% of newly diagnosed bladder cancer is NMIBC (2).

The conventional transurethral resection of bladder tumor (TURBT) is the most common strategy for NMIBC and it is recommended by the guidelines (3, 4). However, the TURBT has a complication rate of ∼4–6%, of which urinary tract infections and significant haematuria are most common (5). In some cases, major complications including obturator nerve reflex (ONR) and bladder perforation could occur. To overcome these drawbacks, lasers including holmium YAG and thulium YAG were introduced. Several studies have suggested the superior safety of Thulium laser resection of bladder tumors (TmLRBT) compared with conventional TURBT (6, 7).

The TmLRBT is increasingly used in the treatment of NMIBC recently, but whether it can provide better cancer control than TURBT is still unclear. Previous studies have compared the recurrence rates of these two therapies but no significant difference was detected (7). However, in all these studies, intravesical therapies were conducted using epirubicin or mitomycin C, instead of bacillus Calmette-Guérin (BCG), which is superior for preventing the recurrence of NMIBC (8–10). Here we retrospectively collected the data of patients who underwent TmLRBT or TURBT followed by BCG therapy to assess the safety and efficacy of these two therapies.

MATERIALS AND METHODS

The study and all its protocols were approved by the institutional review board of the Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Grant number: TJ-IRB20210106). The informed consent was exempted for this retrospective and observational study. All data has been de-identified.

We accessed our institutional database to retrospectively screened all the patients who were treated by conventional TURBT or TmLRBT from August 2018 to December 2019. The inclusion criteria were as follows. (1) Pathologically confirmed as NMIBC. (2) Underwent conventional TURBT or TmLRBT. (3) Intermediate or high risk according to EAU risk stratification (11). (4) Received standard BCG intravesical therapy. (5) With complete clinical and follow-up data. The exclusion criteria were as follows. (1) Locally advanced (T2 or higher), or metastatic bladder cancers. (2) Loss of contact or inadequate clinical information for further analysis. (3) Unable to finish BCG intravesical therapy due to intolerance or other reasons. (4) Comorbidity of other neoplastic diseases.

The medical records of all patients were retrieved and the baseline characteristics were collected. Ultrasonography, intravenous urography, computerized tomography of urinary system (CTU), and cystoscopy were routinely performed before resection to assess the clinical characteristics of the tumors. All the patients chose TmLRBT or TURBT after being informed of the advantages and drawbacks of the two surgical procedures and signed the informed consent. All the surgeries were performed according to standard protocols which have been described in our previous study (12).

If postoperative gross hematuria occurred after surgery, continuous bladder irrigation would be maintained until no sign of postoperative bleeding for 4 h. 30 mg gemcitabine was used for intravesical instillation therapy within 24 h after surgery for the first time.

For patients with intermediate or high risk (11), intravesical BCG therapy would be recommended. Two weeks after that, according to the drug instructions, 2 g BCG in 50 ml of saline was given weekly for 6 weeks, then biweekly for 6 weeks, and then once a month for 10 months. For high-risk patients, monthly intravesical instillations were added for 1–2 years. The ultrasonography and cystoscopy were performed every 3 months for the first 2 years after surgery for recurrence surveillance. An additional telephone follow-up was conducted for patients who performed examinations in local medical institutions.

TABLE 1 | Characteristics of included patients and tumors.

Variable	TmLRBT ($n = 37$)	TURBT ($n = 53$)	*P*-value
Age, year	60.6 ± 9.2	61.2 ± 11.6	0.780
Gender			0.830
Male	30 (81.1%)	42 (79.2%)	
Female	7 (18.9%)	11 (20.8%)	
Previous bladder tumor	9 (24.3%)	6 (11.3%)	0.103
Tumor number	2.9 ± 2.9	2.3 ± 4.0	0.406
Tumor multiplicity			0.520
Single	17 (45.9%)	28 (52.8%)	
Multiple	20 (54.1%)	25 (47.2%)	
Tumor size, cm	1.7 ± 0.8	2.2 ± 1.1	**0.036**
Tumor Location			0.607
Lateral	27 (73.0%)	36 (67.9%)	
Other	10 (27.0%)	17 (32.1%)	
T stage			0.525
Ta	16 (43.2%)	20 (37.7%)	
Tis	3 (8.1%)	2 (3.8%)	
T1	18 (48.6%)	31 (58.5%)	
Tumor Grade (WHO2004)			0.341
PUNLMP	0	2 (3.8%)	
Low Grade	8 (21.6%)	11 (20.8%)	
High Grade	29 (78.4%)	40 (75.5%)	
Risk			
Intermediate	6 (16.2%)	12 (22.6%)	0.453
High	31 (83.8%)	41 (77.4%)	

Data presented as n (%) or mean ± SD.

P-value < 0.05 was considered statistically significant and highlighted in bold.

TmLRBT, Thulium laser resection of bladder tumors; TURBT, Transurethral resection of bladder tumors; TIS, tumor in situ; PUNLMP, papillary urothelial neoplasms of low malignant potential.

TABLE 2 | Intraoperative, postoperative and oncological outcomes.

Variable	TmLRBT ($n = 37$)	TURBT ($n = 53$)	*P*-value
Operative time, min	31.4 ± 17.2	39.4 ± 23.2	0.075
Obturator nerve reflex	0	8 (15.1%)	**0.015**
Bladder perforation	0	3 (5.7%)	0.381
TUR syndrome	0	1 (1.9%)	1.000
Post-operative gross hematuria	16 (43.2%)	51 (96.2%)	**<0.001**
Post-operative irrigation	12 (32.4%%)	50 (94.3%)	**<0.001**
Duration of irrigation*, h	6.5 ± 4.9	24.3 ± 13.7	**<0.001**
Second surgery for hemostasis	0	0	1.000
Post-operative catheterization, d	2.2 ± 0.7	3.1 ± 1.7	**0.002**
Second resection	7 (18.9%)	4 (7.5%)	0.189
Recurrence within 3 months	0	3 (5.7%)	0.381
Recurrence within 1 year	3 (8.1%)	15 (28.3%)	**0.018**

Data presented as n (%) or mean ± SD.
P-value < 0.05 was considered statistically significant and highlighted in bold.
TmLRBT, Thulium laser resection of bladder tumors; TURBT, Transurethral resection of bladder tumors.
**Only the data of patients underwent irrigation was analyzed.*

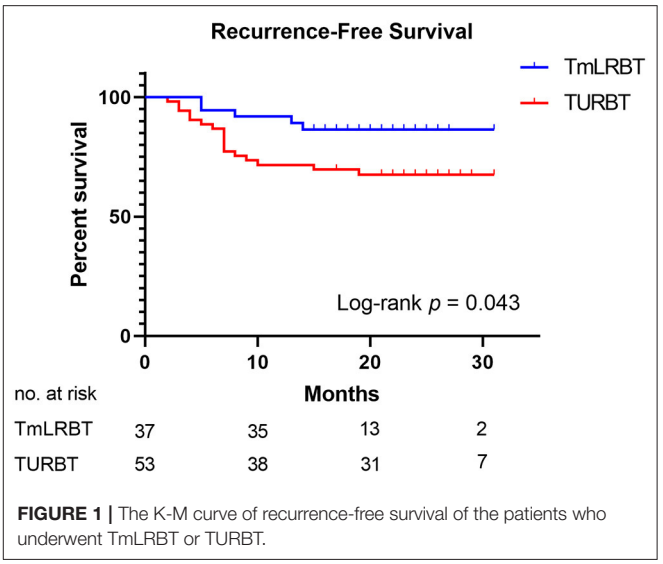

FIGURE 1 | The K-M curve of recurrence-free survival of the patients who underwent TmLRBT or TURBT.

The statistical analysis was conducted using the SPSS 20.0 software. Continuous data were presented as mean ± standard deviation and compared using Student's *t*-test. Categorical data were presented as number (percentage) and compared with the Chi-square or Fisher exact test. Univariate Cox regressions were used to evaluate the predictive role of covariates, including surgery type, age, gender, previous bladder tumor, second resection, tumor number, tumor size, tumor location, pathologic stage, and pathologic grade for recurrence-free survival (RFS). Variables with a *P*-value <0.1 were furtherly included in multivariate Cox regression. The Kaplan-Meier(K-M) curve of RFS was plotted and the log-rank test was conducted with the Graphpad Prism 8.0.1 software.

RESULTS

Eventually, 37 patients who underwent TmLRBT and 57 patients who conducted TURBT group were enrolled in the analysis. As listed in **Table 1**, the baseline characteristics such as age, gender, tumor number, tumor stage, and pathological grade were similar between two groups. The tumor size of the TmLRBT is smaller than that of the TURBT group (1.7 cm vs. 2.2 cm; $P = 0.036$). Nine patients in the TmLRBT group and 6 patients in the TURBT group have a history of bladder tumor and the proportion was comparable in these two groups.

The perioperative results of the two groups are illustrated in **Table 2**. The operation duration of the two groups was similar. During the TURBT, 8 (15.1%) patients encountered ONR, and 3 (5.7%) patients had bladder perforation. Meanwhile, no ONR or bladder perforation occurred during the TmLRBT surgery. After surgery, only one patient in the TURBT group experienced TUR syndrome, and no second surgery for hemostasis was conducted. Postoperative gross hematuria happened in 51 (96.2%) patients in the TURBT group and 16 (43.2%) patients in the TmLRBT group. 50 patients in the TURBT group and 12 patients in the TmLRBT group received postoperative irrigation. Among these patients who underwent postoperative irrigation, the TmLRBT group also has a shorter irrigation duration (6.5 h vs. 24.3 h; $P < 0.001$) than conventional TURBT. Besides, the TmLRBT could shorten the catheterization time (2.2 d vs. 3.1 d; $P = 0.002$).

The RFS graph was illustrated in **Figure 1** and the K-M curve of RFS showed that the TmLRBT group has a longer RFS than the TURBT group (HR: 0.376; 95% CI, 0.162–0.873; Log-rank $P = 0.043$). Univariate and multivariate Cox regressions were conducted to evaluate the predictive value of variates and the results were shown in **Table 3**. As the multivariate Cox analysis suggested, the surgery type (HR: 0.268; 95% CI, 0.095–0.759; $P = 0.013$), history of bladder tumor (HR: 4.319; 95% CI, 1.733–10.769; $P = 0.002$), and pathologic stage (HR: 3.033; 95% CI, 1.023–8.997; $P = 0.045$) are independent predictive factors of RFS. Notably, 3 patients who underwent TURBT experienced recurrence within 3 months after the surgery.

DISCUSSION

Currently, TURBT combined with intravesical therapy is still the "gold standard" for intermediate and high risk NMIBC, and BCG immunotherapy is the recommended adjuvant therapy by guidelines (3, 4). Compared with intravesical chemotherapy including epirubicin and mitomycin C, BCG immunotherapy showed better efficacy in recurrence prevention (9, 10). However, in the previous studies comparing the prognosis of TmLRBT and TURBT, BCG immunotherapy was not applied to these patients (13–18). To the best of our knowledge, the present study is the first research to compare the efficacy of TmLRBT and TURBT under BCG intravesical therapy. In this study, over 90% (89 of 90) patients completed intravesical BCG therapy for 1 year. The completion of each groups was summarized in **Table 4**.

Cancer control is the most critical purpose in the treatment of malignancies. The spreading of tumor cells caused by TURBT

TABLE 3 | Univariate and multivariate Cox analyses of recurrence-free survival.

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Surgery (Ref: TURBT)	0.376 (0.138–1.019)	0.054	0.268 (0.095–0.759)	**0.013**
Second resection (Ref: No)	0.040 (0.001–7.682)	0.229		
Gender (Ref: Female)	0.829 (0.306–2.247)	0.712		
Age (Ref: <70 year)	2.190 (0.807–5.942)	0.124		
Previous bladder tumor (Ref: No)	3.372 (1.407–8.079)	**0.006**	4.319 (1.733–10.769)	**0.002**
Tumor number (Ref: single tumor)	1.517 (0.648–3.551)	0.337		
Tumor size (Ref: <3cm)	0.540 (0.160–1.826)	0.540		
Tumor location (Ref: other)	1.127 (0.441–2.882)	0.803		
Pathologic stage (Ref: Ta)	3.482 (1.177–10.303)	**0.024**	3.033 (1.023–8.997)	**0.045**
Pathologic grade (Ref: PUNLMP and low grade)	2.029 (0.600–6.862)	0.255		
Risk (Ref: Intermediate risk)	2.698 (0.630–11.550)	0.181		

P-value < 0.05 was considered statistically significant and highlighted in bold.
Variables with a p-value < 0.1 in the univariate analysis were included in the multivariate analysis.
TURBT, Transurethral resection of bladder tumors; PUNLMP, papillary urothelial neoplasms of low malignant potential.

TABLE 4 | Summary of the BCG therapy completion.

	TmLRBT	TURBT
Abortion	4 (10.8%)	4 (7.5%)
Completion of 1 year BCG therapy	29 (78.4%)	41 (77.4%)
Completion of 2 year BCG therapy	3 (8.1%)	5 (9.4%)

TmLRBT, Thulium laser resection of bladder tumors; TURBT, Transurethral resection of bladder tumors.

could be a potential reason for recurrence and progression (19–22). During the TmLRBT procedures, the en bloc technique allows a complete enucleation of lesions and avoid tumor fragmentation. Therefore it can potentially minimize the amount of floating tumor cells and diminish the risk of dissemination (23). However, the previous studies did not suggest a significant advantage of TmLRBT in cancer control. In our analysis, after a standard BCG immunotherapy, the TmLRBT showed superior efficacy in the prevention of recurrence than TURBT, which is quite different from the results of most previous studies, in which postoperative intravesical chemotherapy was applied. On the one hand, compared with previous researches, our research included more patients with pathologic high grades (76.7% vs. 10.0% to 30.0%), T1 or Tis stages(60.0% vs. 26.4% to 48.6%), and most patients were high risk (80%) (14–16, 18). The benefit of TmLRBT might be more significant in these tumors with higher risk. On the other hand, the use of BCG immunotherapy might reinforce cancer control of TmLRBT and both treatments synergically suppressed the recurrence. For these tumors with signs of aggressive properties in the preoperative assessments, TmLRBT might be a preferred option.

The complications of TURBT are an essential concern in the treatment of NMIBC. During the TURBT procedure, especially for tumors locating at the lateral bladder wall, the current flow may stimulate the obturator nerve and lead to muscle contraction. In some cases, it could even bring bladder perforation. Several techniques were developed to prevent ONR during the TURBT, including the use of bipolar electrodes and the obturator nerve block. However, the efficacy of bipolar electrodes in the prevention of ONR is still controversial (24–26). As for the obturator nerve block, to achieve a higher success rate, the assistance of ultrasound or nerve stimulator might be needed and the procedure could be time-consuming and complicated. While in the TmLRBT, no current flow was produced and the ONR and bladder perforation could be perfectly avoided.

Another advantage of TmLRBT is the excellent performance in hemostasis. Under the thulium laser, the exposed tissue could be vaporized after being heated to a temperature of 90–100°C.As for the tissue adjacent to the vaporized part, it could be coagulated under 60–80°C (27). The instantly coagulated tissue layer made the hemostasis more efficient. Several studies suggested that TmLRBT was related to a lower postoperative irrigation rate (14) and a shorter irrigation length (15, 16). In our study, fewer postoperative gross hematuria and a lower postoperative irrigation rate in the TmLRBT group were also observed. Even for these patients who need irrigation, the TmLRBT had a shorter irrigation time. Compared with conventional TURBT, the TmLRBT is more feasible for NMIBC.

Notably, thulium laser is not the only laser used in the resection of bladder cancers. Studies had suggested the safety and efficacy of different lasers to treat NMIBC including holmium laser (6), green-light lithium triborate laser (28), and potassium-titanyl-phosphate laser (29). Thulum laser has a wavelength of 2 um, which is nearing the absorption peak of water, therefore it has the most efficient vaporization (30). Another advantage of thulium laser is its shallowest penetration depth compared to holmium and green-light laser (31), which allows more accurate resection and might reduce the risk of bladder perforation.

This present study has several limitations. The limited sample size is the main drawback of this study. As BCG immunotherapy was not widely used in China until recent years, most patients

received maintain chemotherapy such as gemcitabine and epirubicin instead of BCG therapy in the past. Also, the retrospective nature of this study might bring potential bias. For example, in our study, we included these patients who received BCG therapy after surgery, which resulted in a significantly higher proportion of patients with pathologic high grades. The significant oncological outcomes alert us to conduct subgroup analysis in future prospective research to assess the efficacy of TmLRBT. The selection bias during the therapy determination should also be noted. The tumor characteristics could affect the therapy choosing. For example, the TURBT could be often used when handling large size tumors, which could be reflected in the imbalanced tumor size in **Table 1**. Also, the surgeon's preference could also affect the final resection strategy. Besides, as a retrospective study, all cases were collected from the clinical practice. The clinicians managing post-operative care were aware of the surgical methods of each patient. The postoperative parameters such as catheterization and irrigation duration could be biased.

CONCLUSION

In summary, our results suggested that TmLRBT is safer than conventional TURBT with fewer perioperative complications. Besides, TmLRBT could offer better cancer control, therefore might be a superior option for NMIBC patients with intermediate and high recurrence risk. The findings of our study should be ascertained in a further prospective study with larger sample size and longer follow-up.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of the Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology (Grant Number: TJ-IRB20210106). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

ZL and GL wrote this manuscript. YZ, GS, WO, SheW, and HX collected the data. GL analyzed the data. ZW, WG, XY, ZH, and ZC read and edited the manuscript. ShaW and HL designed the study. All authors approved the submitted version.

FUNDING

This work is supported by the Natural Science Fund of Hubei Province (Grant Number: 2018CFB459, Recipient: HL). The funding sources had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, and preparation, review, or approval of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2021.759487/full#supplementary-material

Figure S1 | Diagram for the BCG intravesical therapy schedule.

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frontiers in Surgery

SYSTEMATIC REVIEW
published: 23 November 2021
doi: 10.3389/fsurg.2021.775527

Intravesical Chemohyperthermia vs. Bacillus Calmette-Guerin Instillation for Intermediate- and High-Risk Non-muscle Invasive Bladder Cancer: A Systematic Review and Meta-Analysis

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OPEN ACCESS

Edited by:
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Reviewed by:
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Lazaros Tzelves, National and Kapodistrian University of Athens, Greece

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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *14 September 2021*
Accepted: *27 October 2021*
Published: *23 November 2021*

Citation:
Zhao H, Chan VW-S, Castellani D, Chan EO-T, Ong WLK, Peng Q, Moschini M, Krajewski W, Pradere B, Ng C-F, Enikeev D, Vasdev N, Ekin G, Sousa A, Leon J, Guerrero-Ramos F, Tan W-S, Kelly J, Shariat SF, Witjes JA and Teoh JY-C (2021) Intravesical Chemohyperthermia vs. Bacillus Calmette-Guerin Instillation for Intermediate- and High-Risk Non-muscle Invasive Bladder Cancer: A Systematic Review and Meta-Analysis. Front. Surg. 8:775527. doi: 10.3389/fsurg.2021.775527

Background: The efficacy of intravesical chemotherapy maintenance for patients with non-muscle invasive bladder cancer (NMIBC) is inferior compared to intravesical bacillus Calmette–Guerin (BCG). How intravesical chemohyperthermia (CHT) compares with BCG is under investigation.

Objective: To compare the oncological outcomes and safety profile between intravesical CHT and BCG treatment for intermediate- and high-risk NMIBC.

Methods: We performed a systematic review and meta-analysis of clinical studies comparing CHT with BCG for intermediate- and high-risk NMIBC patients. A comprehensive literature search on OVID MEDLINE, EMBASE, and Cochrane Library was conducted. Risk of bias was assessed by the Cochrane RoB tool and ROBINS-I. Certainty of evidence was rated using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) methodology.

Results: A total of 2,375 articles were identified and five studies were finally included. Among them, four randomised trials comprising 327 patients (CHT group: 156 patients; BCG group: 171 patients) were included in the meta-analysis. There were no significant differences in the 24–36 months recurrence rates (CHT: 29.5%, BCG: 37.4%; RR: 0.83, 95% CI 0.61–1.13; moderate certainty of evidence) and the 24–36 months progression rates (CHT: 4.4%, BCG: 7.6%, RR = 0.62, 95% CI 0.26–1.49; low certainty of evidence). There were also no significant differences in grade 1–2 adverse events (CHT group: 59.9%, BCG group 54.5%; RR = 1.10, 95% CI 0.93–1.30; moderate certainty of evidence) and grade 3 or above adverse events (CHT group: 23.2%, BCG group 22.5%; RR = 0.99, 95% CI 0.69–1.43; low certainty of evidence).

Conclusions: Intravesical CHT had equivalent oncological outcomes and similar safety profile when compared to BCG maintenance therapy for patients with intermediate- and high-risk NMIBC. CHT is a possible alternative treatment in the times of BCG shortage.

Keywords: bladder cancer, TURBT (trans-urethral resection of bladder tumour), BCG–Bacillus Calmette-Guérin vaccine, chemohyperthermia, meta-analysis

INTRODUCTION

Bladder cancer is the 11th most common cancer worldwide, and more than 75% of the patients present with non-muscle invasive bladder cancer (NMIBC) (1, 2). Transurethral resection of bladder tumour (TURBT) is a potentially curative surgery, yet the oncological control of NMIBC is unsatisfactory with a one-year recurrence rate of up to 31%, and a five-year recurrence rate of up to 78% (3, 4).

NMIBC is classified into low-risk, intermediate-risk, and high-risk disease based on its clinical and pathological factors (5, 6). For intermediate- and high-risk NMIBC, intravesical bacillus Calmette–Guerin (BCG) therapy has been shown to be effective in reducing disease recurrence and progression (7). On the other hand, intravesical BCG therapy is associated with local and systemic toxicities, and it may not be well-tolerated throughout the whole treatment course (8, 9). Moreover, BCG shortage is a significant global problem (10). There is an urgent need to seek for an alternative treatment that is at least equally effective, and with better tolerability and secured availability for patients with intermediate- and high-risk NMIBC (11).

Intravesical maintenance chemotherapy has long been investigated in patients with NMIBC. Although it was associated with a lower rate of adverse events, its treatment efficacy has been proven to be inferior to intravesical maintenance BCG therapy (12, 13). In recent years, there has been increasing use of adjuvant intravesical chemohyperthermia in NMIBC patients. By increasing the temperature of chemotherapy (Combat System) or the bladder wall (Synergo system) to 42–43°C degrees, may enhance its drug absorption and cytotoxic effects (14). Although intravesical CHT is a promising treatment, its distinction of treatment outcome comparing with BCG is not well-known. In this systematic review, we aim to investigate the treatment efficacy and adverse events of intravesical CHT vs. BCG in patients with intermediate- and high-risk NMIBC.

METHODS AND MATERIALS

A systematic review and meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (15). The study protocol was registered on the international prospective register of systematic reviews (PROSPERO) (Registration number: CRD42020223277).

Literature Search

We conducted a comprehensive literature search on OVID MEDLINE, EMBASE, and Cochrane Central Controlled Register of Trials (CENTRAL), using Medical Subject Headings (MeSH) terms and keywords related to "Bladder cancer," "Bacillus Calmette-Guérin," and "Chemohyperthermia." The search was performed from database inception up to the 1st of September 2020. All full-text publications, conference abstracts and proceedings in English language were included. Reference lists of the included studies were sought for additional articles. The search strategy is presented in **Supplementary Material**.

Selection Criteria

Randomised controlled trials (RCTs) and observational studies comparing the use of CHT and BCG instillation in intermediate- or high-risk NMIBC patients post-TURBT were included. Only human studies were included and there was no limit to the type of CHT device used. Editorials, commentaries, reviews, case reports, case series and single arm studies were excluded. Studies comparing the use of CHT and normothermic chemotherapy were also excluded.

Screening and Data Extraction

All identified articles were initially screened by two independent reviewers by title and abstract. Conflicts were resolved by a third senior author. Full texts of potentially eligible studies were then retrieved for further screening in the same manner.

Finally, a standardised and piloted data extraction form was devised to capture data such as baseline characteristics of studies, details of intervention and control, along with outcomes of interest. The corresponding authors of each study with missing data were contacted in order to retrieve any missing data.

Data Synthesis and Statistical Analysis

The primary outcomes of our study included recurrence and progression rates at 24–36 months. Secondary outcomes included recurrence-free survival (RFS), progression-free survival (PFS), grade 1–2 and grade 3 or above adverse events (AEs) according to the National Cancer Institute Common Terminology Criteria (16). Meta-analyses were only performed when there were two or more RCTs reporting the same outcome under the same definition. Rates of recurrence, progression and AEs were analysed as dichotomous events using the Mantel-Haenszel method, and were reported as risk ratios (RR), 95% CIs and *p*-values. For RFS and PFS, hazard ratios (HR) and 95% Confidence Interval (95% CI) derived by the Cox Proportional hazards model were pooled using the inverse variance method, and were reported as HRs, 95% CIs and *p*-value. In studies where Hazard Ratios were not reported, HRs are estimated using validated methods outlined by Tierney et al. (17) as recommended by the Cochrane Collaboration (18). The random effects (RE) model was used to take into account substantial heterogeneity where identified, otherwise, the fixed effects (FE) model was used. Heterogeneity was assessed using the Cochran's I^2, and substantial heterogeneity was defined as an I^2 value >50% or a Chi^2 *p*-value <0.10. Owing to the potential source of heterogeneity originating from the types of CHT used, subgroup differences were tested between the major types of CHT used, and was defined as a Chi^2 *p*-value <0.10. Planned sensitivity analyses were also performed on patients without BCG failure and without carcinoma *in situ* (CIS) diseases. All data-analyses were performed using Review Manager v.5.4. Results from non-randomised studies were summarised narratively. Risk of bias of RCTs was assessed using the Risk of Bias 2.0 tool as recommended by the Cochrane Collaboration (19, 20). Risk of bias in non-randomised studies were assessed using the non-randomised studies–of interventions (ROBINS-I) tool (21). Summary of findings for all outcomes, along with the certainty of evidence which was rated according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach (22) were tabulated using the GRADEpro tool (23).

RESULTS

Literature Search Results

The PRISMA flow diagram is shown in **Figure 1**. A total of 2,956 records were identified upon the literature search. No additional records were identified during screening of reference lists of included articles. 2,361 abstracts remained after removal of duplicates. A total of 2,277 articles were excluded upon initial screening, and 79 studies were further excluded upon full-text screening. Finally, four RCTs (24–27) were included for meta-analysis, and one observational retrospective study (28) was retrieved and included for qualitative synthesis. Two studies included both intermediate- and high-risk NMIBC patients (24, 25), and the other three studies included high-risk NMIBC patients only (26–28). All studies were non-inferiority trials and did not specifically focus on primary or recurrent cases. All five studies had similar follow-up durations of 24–36 months. The study information of the five studies is shown in **Table 1**. The risk of bias assessment and the GRADE summary of finding profiles are included in **Supplementary Material**.

Study Outcomes

Recurrence Rate at 24–36 Months

Four RCTs with 327 patients were included (24–27). There was no significant difference between the two groups (RR_{FE} 0.80, 95% CI 0.59–1.08; moderate certainty of evidence) (**Figure 2**). No significant heterogeneity ($I^2 = 1\%$, $p = 0.38$) was detected. Upon subgroup analysis, no differences were found between the use of conductive hyperthermia and radiofrequency-induced thermochemotherapeutic effect (RITE). Sensitivity analysis after excluding BCG failure patients from the HYMN study shows CHT has a significantly lower recurrence rate when compared to BCG group (RR_{FE}: 0.64, 95% CI 0.42–0.98, $p = 0.04$) (**Supplementary Material**). Of note, in the RCT by Sousa et al. (26), the conductive CHT group had significantly lower rate of recurrence when compared to the BCG group (20.5% vs. 38.2%, $p < 0.02$). However, a retrospective matched cohort study by Ekin et al. (28) found a significantly higher recurrence rate in patients receiving conductive CHT compared to those who received BCG (35.9% vs. 20.5%, $p < 0.05$).

Progression Rate at 24–36 Months

Four RCTs with 327 patients were included (24–27). There was no significant difference between the two groups (RR_{FE} 0.60, 95% CI 0.26–1.41, $p = 0.24$; low certainty of evidence) (**Figure 3**). No significant heterogeneity was detected ($I^2 = 0\%$, $p = 0.67$). No significant difference between conductive hyperthermia and RITE was found upon subgroup analysis. When excluding patients with BCG failure, progression rate was also found to be similar in CHT patients when compared to BCG patients (RR_{FE} 0.38, 95% CI 0.12–1.22, $p = 0.10$). Of note, in the RCT performed by Sousa et al. (26), T1 progression and T2 progression were significantly reduced in the conductive system CHT group when compared to the BCG group ($p < 0.05$ and $p < 0.01$ respectively). However, in a retrospective matched cohort study performed by Ekin et al. (28) the use of conductive CHT was associated with significantly higher progression rate when compared to BCG (15.4% vs. 7.7%, $p < 0.05$).

Recurrence-Free Survival

Three RCTs were included in the meta-analysis (24, 25, 27). In terms of RFS, no significant difference was noted between the CHT group and the BCG group (HR_{RE} 0.81, 95% CI 0.42–1.56, $p = 0.53$; very low certainty of evidence) (**Supplementary Material**). However, there was significant heterogeneity amongst the included studies ($I^2 = 68\%$, $p = 0.04$).

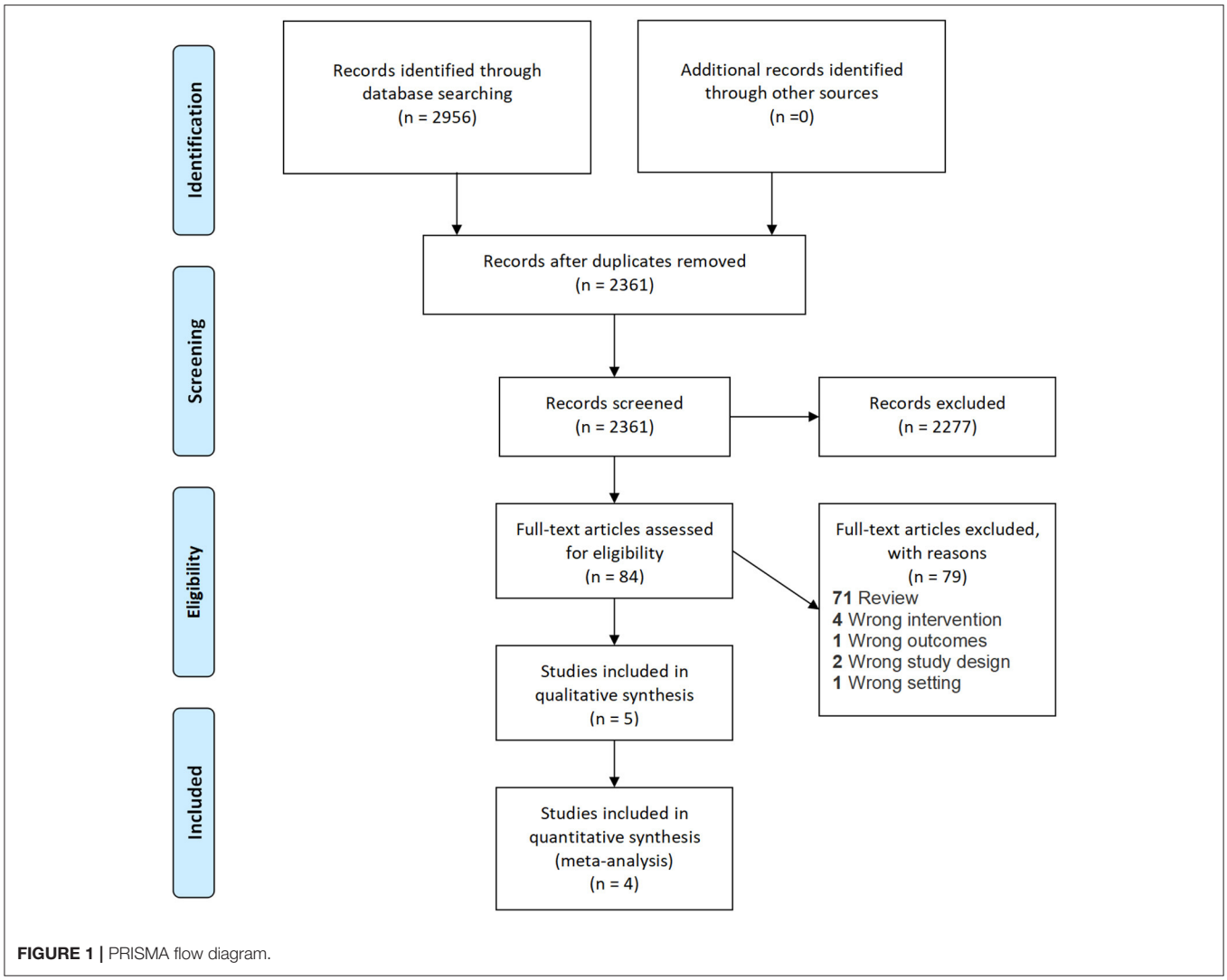

FIGURE 1 | PRISMA flow diagram.

Our subgroup analysis suggested that this heterogeneity did not originate from the type of CHT systems used; no differences were found between the conductive CHT group and the SRITE group. When performing a sensitivity analysis to exclude patients with BCG failure (i.e., patients from the HYMN trial), RFS is found to be significantly better in CHT patients than BCG patients (HR_{RE} 0.57, 95% CI 0.33–0.98) with no significant heterogeneity ($I^2 = 0\%$, $p = 0.91$) (**Supplementary Material**), suggesting the potential source of heterogeneity to originate from BCG failure patients. Of note, in a retrospective study by Ekin et al. (28), it was found that the use of conductive CHT was associated with significantly worsened RFS when compared to BCG instillation (HR 4.18, 95% CI 1.37–12.71, $p = 0.012$). However, when performing sensitivity analysis by excluding the HYMN study, where only patients with BCG failures were considered, the remaining two studies show that CHT group has a better RFS when compared to the BCG group (HR_{FE} 0.52, 95% CI 0.29–0.93; **Supplementary Figure 1**). However, when excluding patients with CIS disease, the RFS is both groups remained similar (HR_{FE} 0.72, 95% CI 0.48–1.09) (**Supplementary Material**).

Progression-Free Survival

Two RCTs were included in the meta-analysis (24, 27). In terms of PFS, there was no significant difference between the CHT group and the BCG group (HR_{RE} 0.92, 95% CI 0.25–3.40; very low certainty of evidence) (**Supplementary Material**). However, there was significant heterogeneity amongst the included studies ($I^2 = 73\%$, $p = 0.06$). The heterogeneity might originate from the different types of CHT device being used as evident by the test for subgroup differences ($p = 0.06$), but this should be interpreted with caution due to the limited number of studies being included. Furthermore, the study by Tan et al. also included BCG failure and CIS patients, which may have lead to a significantly lower PFS rate. In the retrospective study by Ekin et al. (28), no significant difference was found between the CHT group and the BCG group (HR 1.72, 95% CI 0.28–10.36, $p = 0.550$).

TABLE 1 | Characteristics of the included studies.

Study (year)	Country of study	Study design	Number of centres	Recruitment period	Duration of follow up (months)	Inclusion and exclusion criteria	Number of patients (Intervention/ control)	Age (intervention/ control)	Sex (M/F)	Device used	Regime for CHT	Regime for BCG
Sousa 2020	Spain	RCT	2	Between March 2015 and June 2019	Mean: 38	1. Histological confirmed previous UCC 2. NMIBC following recurrence of G1-3 pTa or G1-2 pT1 3. Tumour number ≤6 number of tumours 4. Aged ≥18 years 5. No solid tumour, muscle infiltrating aspect or CIS suspicious, positive cytology and recurrence of previous T1G3 or CIS tumours in the last 12 months	16/17	Mean ± SD: 71 ± 3.2/69 ± 2.7	27/6	Combat BRS system	Weekly for 8 weeks, 80 mg MMC	NA
Guerrero-Ramos 2020	Spain	RCT	1	NR	Median: 24.8	1. NMIBC 2. No CIS 3. No intolerance or contraindication for receiving BCG or MMC	24/24	Entire group mean: 73	42/6	Combat BRS system	Weekly for 6 weeks; follow by monthly for 6 months, 40 mg MMC	Weekly for 6 weeks and maintenance according to SWOG protocol.
Ekin 2015	Turkey	Retrospective cohort study	2	Between January 2004 and January 2014	Median(IQR): 33(24–39)	1. High-risk of NMIBC treated with intravesical C-HT or BCG instillation 2. Performed second-TUR 3. Not treated with reduced dose of BCG, 4. No bladder diverticulum >1 cm 5. No histopathology non-urothelial carcinoma 6. No concomitant urothealial carcinoma in the urethra or upper urinary tract 7. No low bladder capacity (<150 mL) 8. No high post-voided residual urine (>100 mL)	39/39	Mean ± SD (range): 68.05 ± 9.29 (47–84)/ 68.02 ± 8.42 (48–82)	73/5	Elmedical technologies BWT	Weekly for 6 weeks; Also 3 weekly instillations at month 3 and month 6. 40 mg MMC	Weekly for 6 weeks. The choice of maintenance was determined by the physician and/or patient.

(Continued)

TABLE 1 | Continued

Study (year)	Country of study	Study design	Number of centres	Recruitment period	Duration of follow up (months)	Inclusion and exclusion criteria	Number of patients (Intervention/ control)	Age (intervention/ control)	Sex (M/F)	Device used	Regime for CHT	Regime for BCG
Arends 2016	Israel Italy, the Netherlands Austria, France, Belgium	RCT	11	Between 18 July 2002 and 25 December 2011	Median(range): 25.6 (0.0–34.0)	1. pT1 or grade3 UCC and/or CIS or multifocal (six or more) pTa lesions and/or multiple (three or more) recurrences of pTa lesions in the last 24 months 2. WHO performance status ≤2, 3. Life expectancy >24 months 4. No histopathology non-urothelial carcinoma (basal cell carcinoma excluded) 5. No UCC involving the urethra or upper urinary tract 6. No previous history of UCC stage T2 or higher 7. No intravesical MMC treatments during the previous 12 months 8. No previous BCG therapy <48 mo 9. No previous pelvic radiotherapy, systemic chemotherapy or partial cystectomy 10. No bladder diverticulum >1 cm, residual urine >100 ml, bladder volume <150 ml, urinary incontinence, urethral stricture impeding 20F catheterization 11. No persistent haematuria 12. No active intractable or uncontrollable UTI, active tuberculosis or BCG infection	89/95	Mean ± SD: 65.2 ± 10.67/ 67.4 ± 10.08	154/30	Synergo system	Weekly for 6 weeks, followed by 6 maintenance sessions at 6-wk intervals during the rest of year 1. Two 30-min treatments with 20 mg MMC	Six weekly induction sessions and three weekly repeated maintenance sessions at months 3, 6, and 12

(Continued)

TABLE 1 | Continued

Study (year)	Country of study	Study design	Number of centres	Recruitment period	Duration of follow up (months)	Inclusion and exclusion criteria	Number of patients (Intervention/ control)	Age (intervention/ control)	Sex (M/F)	Device used	Regime for CHT	Regime for BCG
						13. No previous BCG life-threatening sepsis, MMC or BCG allergy, impaired immune response, positive HIV serology, receipt of systemic steroids or immunosuppressives 14. No haematological disorders, leukocytes <3500, platelets <100 000, kidney or liver function disorders (>1.5 times upper normal limit), and pregnant/lactating.						
Tan 2019	UK	RCT	14	Between May 2010 and July 2013	Median:36	1. Recurrence of intermediate- or high-risk NMIBC following induction/maintenance BCG 2. Having complete TUR of papillary lesions 3. pT1 disease underwent re-resection to confirm the absence MIBC 4. Age ≥18 years 5. WHO performance status ≤4 6. Unfit or unwilling to have radical cystectomy 7. Imaging showed no upper tract disease ≤12 mo. 8. Haematological and biochemical blood tests were within normal limits 9. No non-urothelial carcinoma 10. No low-grade NMIBC recurrence	48/56	Median (IQR) 77 (72–82)/76 (67–81)	78/26	Synergo system	Weekly for 6 weeks Patients who were disease -free 3 mo after treatment commencement would proceed to maintenance RITE (one instillation of RITE every 6 wk for 1st yr and one instillation every 8 wk for 2nd yr). Two 30-min cycles, each with 20 mg MMC	Six consecutive weekly instillations followed by maintenance therapy (three consecutive weekly instillations at 3, 6, 12, 18, and 24 mo)

(Continued)

TABLE 1 | Continued

Study (year)	Country of study	Study design	Number of centres	Recruitment period	Duration of follow up (months)	Inclusion and exclusion criteria	Number of patients (Intervention/ control)	Age (intervention/ control)	Sex (M/F)	Device used	Regime for CHT	Regime for BCG
						11. No treatment with intravesical chemotherapy ≤6 mo (single post-TUR instillation allowed) 12. No prostatic urethra or upper tract disease 13. No MMC allergy 14. No active/intractable urinary tract infection 15. No urethral stricture, small bladder capacity (<250 ml), significant urinary incontinence, or history of pelvic radiotherapy.						

WHO, World Health Organisation; NR, not reported; UCC, urothelial cell carcinoma; NMIBC, non-muscle invasive bladder cancer; CIS, Carcinoma in situ; MMC, mitomycin C; BCG, bacillus Calmette-Guerin; UTI, urinary tract infection; TUR, transurethral resection.

Adverse Events

Four RCTs with 368 patients were included (24–27). For Grade 1-2 AEs, there was no significant difference between the CHT group and the BCG group (RR_{FE} 1.11, 95% CI 0.93–1.32, $p = 0.26$; moderate certainty of evidence), and no significant heterogeneity was detected ($I^2 = 0\%$, $p = 0.96$) (**Figure 4**). For grade 3 of above AEs, there was also no significant difference between the CHT group and the BCG group (RR 1.02_{FE}, 95% CI 0.711.47, $p = 0.92$; low certainty of evidence), and no significant heterogeneity was detected ($I^2 = 0\%$, $p = 0.69$) (**Figure 5**).

DISCUSSION

Intravesical BCG therapy is a standard treatment for patients with intermediate- and high-risk NMIBC following TURBT (5). However, it is not without limitations. First, more than half of the patients might develop local and systemic toxicities, such as bacterial/chemical cystitis, frequency, haematuria, allergic reactions and BCG sepsis (8, 9, 29). While a minimum duration of 1-year treatment course is recommended, about half of the patients would withdraw from treatment prior to completion of BCG therapy (30, 31). Second, the supply of BCG has been unsteady in the past decade. Globally, there were only a few manufacturers of BCG, and the production of BCG is generally limited by the slow growth of mycobacteria (10). Therefore, it is imperative for researchers to look for alternative treatments for patients with intermediate- and high-risk NMIBC.

Intravesical chemotherapy has been proven to be less effective than BCG (12, 13). However, the development of device-assisted technology could optimise the efficacy of chemotherapy and potentially maintaining its safety and tolerability. In particular, CHT has gained significant traction within the urological community leading to a steadily increasing use in the past decade. The cytotoxicity of chemotherapy can be accentuated when its temperature reaches 42 to 43 degrees (32). Several mechanisms of action of hyperthermia has been postulated to synergistically enhance the efficacy of intravesical chemotherapy. First, hyperthermia alone could cause the denaturation of cytoplasmic structures and enzymatic proteins, thus inducing cell death by apoptosis and necrosis (32–34). Second, temperature elevation could enhance the permeability of cell membrane and improve drug absorption (35, 36). Third, heat shock proteins could be released upon hyperthermia, thus stimulating an adaptive T cell response to induce both innate and adaptive immune system. Tumour chemosensitization may also be achieved via the heat shock proteins-mediated pathways (37, 38).

Delivery of hyperthermia can be achieved by two main methods, namely conductive hyperthermic chemotherapy (Combat system) and RITE (Synergo). For conductive hyperthermic chemotherapy, the chemotherapy solution was heated externally and recirculated to the bladder at a constant temperature. For RITE, microwave radiation was delivered to the bladderwall at a frequency of 915 MHz. Without the need of conductive delivery of energy, it has a potential benefit to penetrate low-conductive tissues (39).

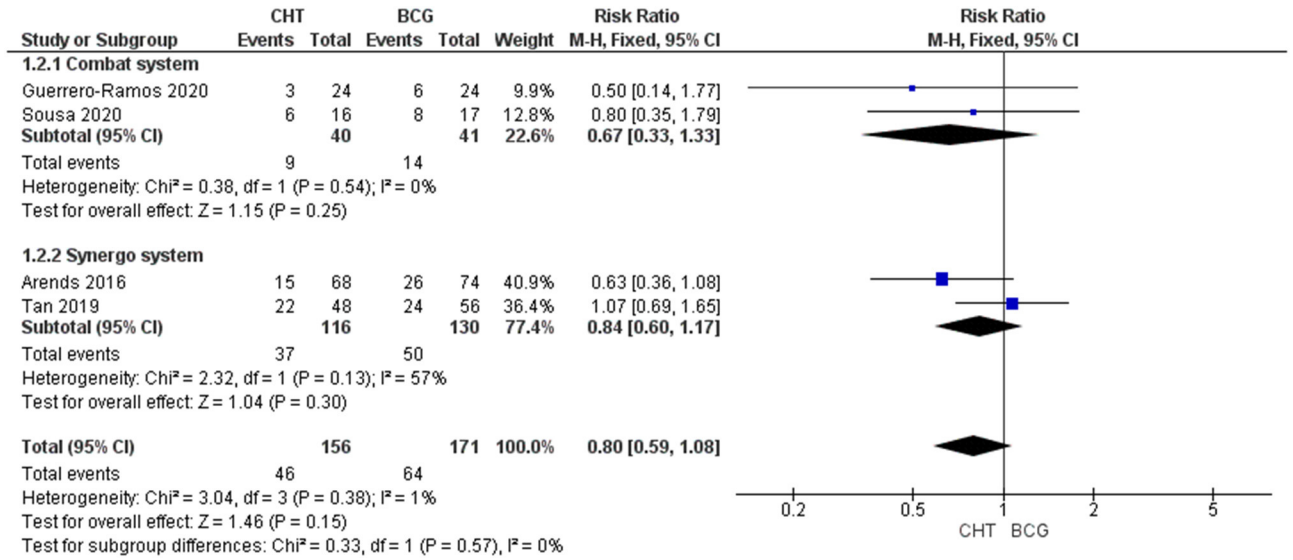

FIGURE 2 | 24–36 months recurrence rate stratified by the type of chemohyperthermia device.

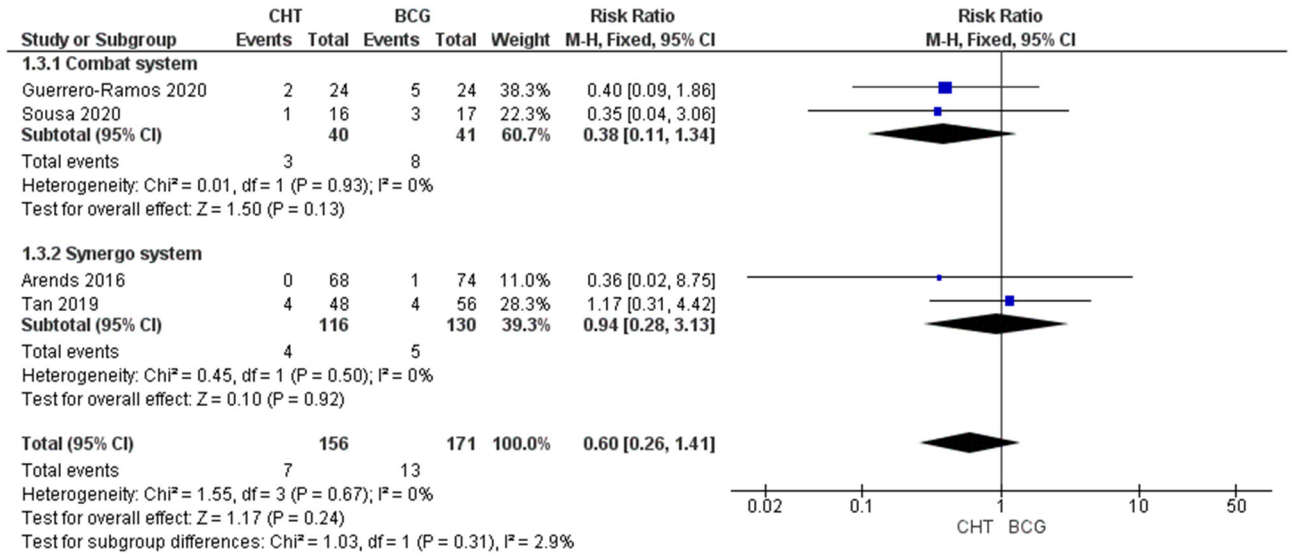

FIGURE 3 | 24–36 months progression rate stratified by the type of chemohyperthermia device.

In our study, we compared between intravesical CHT and BCG in patients with intermediate- and high-risk NMIBC. Our results showed that CHT could achieve an equivalent oncological outcome as BCG therapy in terms of recurrence and progression rates at 24–36 months. Our sensitivity analysis would suggest that efficacy was generally consistent across the two different types of CHT technologies, the Combat/ Unithermia system and the Synergo system. One study population was, however, too small to allow statistically powered comparison between the CHT devices. Intravesical CHT is a reasonable treatment option for intermediate- and high-risk NMIBC given its similar efficacy to BCG. Although the use of CHT was associated with additional costs, a more steady supply can be assumed without the worry of BCG shortage. On the other hand, our meta-analysis showed that the rates of grade 1–2, and grade 3 or above AEs were similar between intravesical CHT and BCG. In other words, based on the current evidence, we cannot assume that CHT is safer or more tolerable than BCG therapy. A realistic expectation should be given when we counsel patients on the usage of CHT.

In many parts of the world, intravesical maintenance chemotherapy is the mainstay of treatment for intermediate-risk, and even high-risk NMIBC (40). A recent meta-analysis

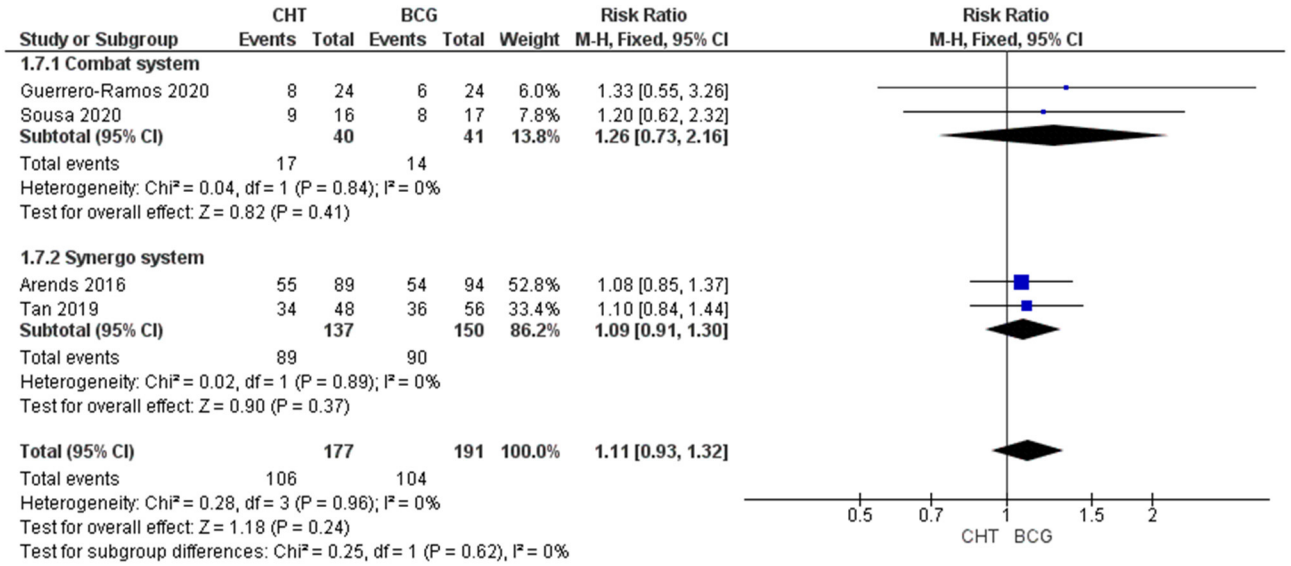

FIGURE 4 | Grade 1–2 adverse events stratified by the type of chemohyperthermia device.

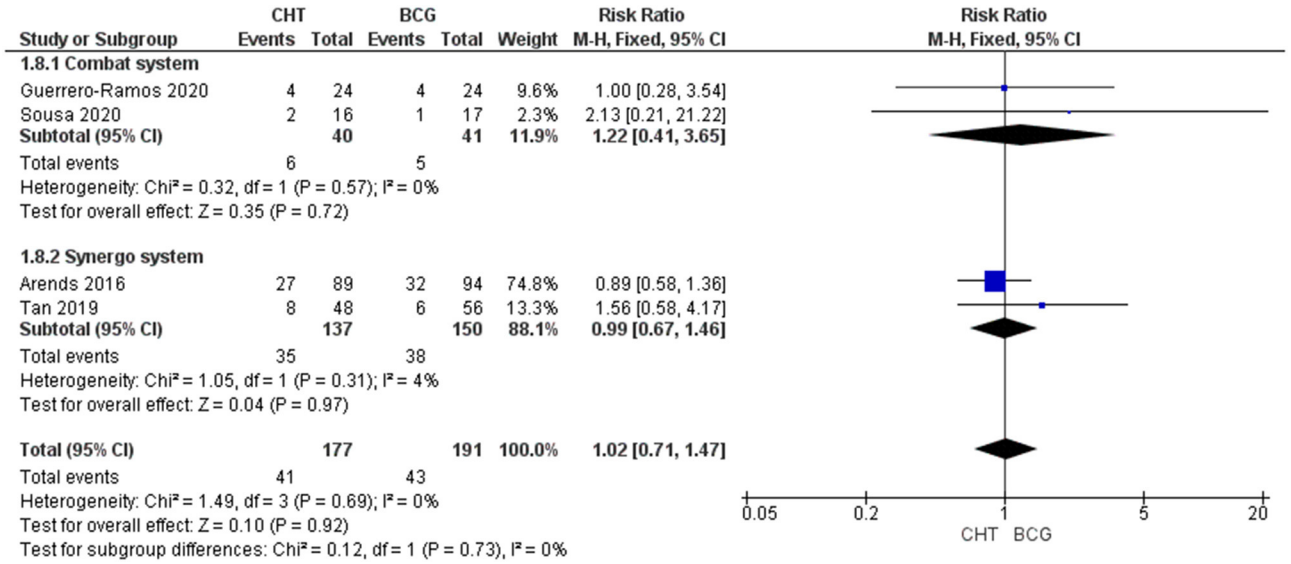

FIGURE 5 | Grade 3 or above adverse events stratified by the type of chemohyperthermia device.

showed that intravesical CHT was associated with a lower recurrence rate when compared to normothermic chemotherapy. The HIVEC I and HIVEC II studies are both multicentre RCTs comparing between CHT and normothermic chemotherapy in patients with intermediate-risk NMIBC. Initial results on safety and tolerability were comparable between the two groups (41); the final oncological outcomes are eagerly awaited.

To our knowledge, this is the first meta-analysis comparing between CHT and BCG in patients with NMIBC. It is based on a comprehensive literature search including conference abstracts and proceedings, therefore publication bias is minimised. Only data from RCTs were meta-analysed, and the certainty of evidence was determined using the GRADE methodology. On the other hand, there are several limitations in our study. First, only four RCTs were included and the sample size is still relatively limited. More RCTs comparing intravesical CHT to BCG are warranted. Second, some of the included RCTs are still on-going, so the collected data may be premature and may not be reflective of the final results. Third, significant heterogeneity does exist in some of our analysis. This may be due to the differences in the underlying patient cohort characteristics; the results should be therefore interpreted with caution. Further sources of

heterogeneity may have been from the definition of high-risk bladder cancer, contributed by the recent change in guidelines as well as potentially different treatment regimens between studies. Finally, while carefully considered using sensitivity analyses, design studies incorporating CIS or papillary disease patients or BCG failure patients may be additional sources of heterogeneity. Nevertheless, our study did shed light on the utility of CHT in patients with intermediate- and high-risk NMIBC. Compared to BCG therapy, intravesical CHT could be an equally effective and tolerable treatment option. Although the utility of CHT implies additional cost, a more "comfortable" treatment regime for patients with a shorter overall treatment time may be preferred. Utility of CHT may also provide a solution to the problem of BCG shortage worldwide. The results have important implications in our clinical practise until higher level of evidence arises.

CONCLUSION

Our meta-analysis showed that intravesical CHT had equivalent oncological outcomes and similar safety profile when compared to BCG therapy for patients with intermediate- and high-risk NMIBC. In well-selected patients, i.e., those without BCG failure, CHT is even more superior than BCG maintenance in terms of recurrence rate. Intravesical CHT is a possible alternative treatment in the times of BCG shortage. More RCTs comparing intravesical CHT to BCG are warranted to develop a clearer image of the value-based utility of CHT in this patient population.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

HZ and VC: data collection, interpretation of data, and manuscript writing. DC, EC, and C-FN: interpretation of data. WO and QP: data collection. MM, WK, BP, DE, NV, GE, AS, JL, FG-R, W-ST, JK, SS, and JW: raw data providing. JT: data collection, interpretation of data, manuscript writing, and supervising. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsurg.2021.775527/full#supplementary-material

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Conflict of Interest: JK and W-ST are investigators of the HYMN trial where SYNERGO provided the system and catheters, SYNERGO was not involved in the conception, design and execution of the trial. The HYMN trials was funded by the Cancer Research UK. JK and W-ST are consultants to Combat Medical. FG-R is an advisor and speaker for Combat Medical.

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frontiers in Surgery

REVIEW
published: 02 December 2021
doi: 10.3389/fsurg.2021.754741

Bladder Cancer Sample Handling and Reporting: Pathologist's Point of View

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The aim of this narrative review is to provide adequate information on handling and reporting of the bladder cancer samples to improve the closely collaboration between pathologists and urologists. The main (but not exclusive) research tool used was PubMed and 87 references were selected and quoted in the text. We have considered handling of biopsies, transurethral resection (TUR), and cystectomy specimens to summarize the different methods of sampling and the related issues. Moreover, we considered and discussed the main prognostic factors, such as histological tumor type, grade, and stage of bladder cancer, that should be described in the pathological report. In addition, critical issues encountered in the interpretation of histological samples were discussed.

Keywords: bladder, carcinoma, cystectomy, staging, handling, reporting

OPEN ACCESS

Edited by:
Jeremy Teoh, The Chinese University of Hong Kong, Hong Kong SAR, China

Reviewed by:
Petros Sountoulides, Aristotle University of Thessaloniki, Greece
Richard Naspro, Fondazione Ospedale San Matteo (IRCCS), Italy

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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: *06 August 2021*
Accepted: *15 October 2021*
Published: *02 December 2021*

Citation:
Mazzucchelli R, Marzioni D, Tossetta G, Pepi L and Montironi R (2021) Bladder Cancer Sample Handling and Reporting: Pathologist's Point of View. Front. Surg. 8:754741. doi: 10.3389/fsurg.2021.754741

INTRODUCTION

A close collaboration between urologist and pathologist is essential for accurate diagnosis and management of patient with bladder cancer. The decision-making for the treatment of bladder cancer depends on both quality of surgical specimen and accuracy of the pathological diagnosis. The precise description of clinical history and an adequate urological information, such as bladder lesion cystoscopic or tomographic scan appearance, timing, surgical or therapeutic procedure performed allow pathologist to decide the best approach in handling and processing the surgical specimens, so as to obtain an accurate pathology report (1–5). The reports can present some differences on the basis of the surgical specimens: for example, report on transurethral resection (TUR) specimens supplies the main information that determines subsequent patient management, such as re-TUR or radical treatment, while report on cystectomy may have an effect on further adjuvant chemo/radiotherapy or appropriate surveillance. In this review, we have considered handling of biopsies, TUR, and cystectomy specimens to summarize the different methods of sampling and the related issues. Moreover, the main prognostic factors, such as histological tumor type, grade, and stage of bladder cancer, that should be described in the pathological report were considered and discussed to make understandable terminology and histopathological problems to urologists (6, 7). The aim of this narrative review was to provide practical points for pathologists and urologists concerning the above described trans-disciplinary topics. The main (but not exclusive) research tool was PubMed. The key words used were "bladder cancer or bladder carcinoma," in addition to various combinations of stages, grades, variants, lympho-vascular invasion, handling, pathological report, and histopathological report. Collateral research included "histochemistry and smoothelin" and "histoanatomic variance and bladder." The cited

articles were mostly published between 2009 and 2021. A number of 87 references were selected and quoted in the text. The data were organized in chapters reflecting the current status of bladder cancer handling and reporting.

CLINICAL INFORMATION

The urologists play a main role in uropathology practice not only as responsible for providing adequate tissue samples for pathological evaluation but also giving useful clinical information to the pathologist to decide the best approach in handling and processing the surgical specimens and draw up an accurate pathology report (1, 3–5).

The urologist should indicate:

- demographic information and clinical history of the patient, bladder cytology if present, whether it is the first presentation of the tumor and if not, details of previous resection;
- the cystoscopic appearance of bladder mucosa and indicate number, size, location of the tumor/s, the morphological features of the lesion: papillary, solid, or ulcerate;
- the state of remaining mucosa if further biopsies were performed;
- if previous radiotherapy to the bladder or to adjacent organ were performed;
- if after the first transurethral resection of bladder tumor (TURBT) local treatments, such as bacillus Calmette-Guérin (BCG) or Mitomycin C intravesical instillation, were performed.

This information is necessary for a correct evaluation of urothelium because the treatments can have an impact on tumor morphology and on normal-looking urothelium in the samples obtained from both re-TURBT or cystectomy (7).

In case of cystectomy, the urologist should provide further information, such as (2, 3):

- information about previous surgical treatments, location, and pathological diagnosis of bladder lesion/s;
- cystoscopic appearance of the bladder mucosa;
- tomographic scan or MRI of the bladder (if performed) to better compare them with macroscopic appearance of the specimen;
- information concerning neoadjuvant chemotherapy, location, number, and size of the lesion/s presents in bladder before therapy to avoid the difficulties in identifying the tumor/s.

SPECIMEN HANDLINGS

Biopsy

Biopsies of the bladder can be taken through cystoscope using cold cup forceps, diathermy forceps, or small diathermy loop (8). Biopsy specimen obtained by cold cup forceps does not show artifacts because a Bugbee electrode is used later to cauterize the urothelial defect (9). Tissue bladder biopsy may be obtained using a resectoscope but this procedure is more invasive and a biopsy specimen can show altered histologic characteristics secondary to the effects of electrical coagulation of tissue (8).

The bladder cold cup biopsy is usually 2–3 mm in diameter, it could contain up to the superficial part of muscularis propria (MP) depending on anatomical part of bladder and on operator skill. The biopsy specimens should be wholly paraffin embedded for histological examination. The biopsy specimens can show small papillary neoplasms, erythematous, or velvet area of urothelium that can represent carcinoma *in situ* (CIS) and/or inflammation. Cold cup biopsy mapping of normal-looking mucosa is not in routine use but this approach is recommended for patients with positive urine cytology and negative cystoscopy or a history of high grade non muscle invasive bladder cancer or in tumors with non-papillary appearance (4, 5, 10, 11). To obtain representative mapping of the bladder mucosa, biopsies should be taken from trigone, bladder dome, and right, left, anterior posterior bladder walls. A specimen of urethra may be useful to assess the extension of disease.

Then, these biopsies should be put in separate jars and subsequently paraffin embedded in different blocks. At least tissue sections at three different levels for each biopsy need for histological evaluation. Deeper levels are recommended if the urothelium surface is not wholly visible and to find suitably orientated urothelium (4, 5).

TUR Specimens

Transurethral resection of bladder tumor is the gold standard for the treatment of non-muscle invasive bladder cancer larger than 6 mm. Tumors $\leq$1 cm as larger size can be resected "en bloc" during TUR procedure (11, 12). En bloc resection is an emerging surgical technique that provides a circumferential incision of the bladder mucosa at a safety margin of few millimeters from the lesion. This technique allows removing the whole tumor, such as the underlying detrusor muscle. Several energy sources are used for this surgical technique, such as monopolar or bipolar current, Holmium and Thulium laser, and hydrodissection (11, 13). Recent studies have demonstrated that "en bloc" resection of bladder tumor (ERBT) should be considered feasible for bladder tumor size of $\leq$3 cm (14–16). The technical limits for ERBT concern mainly the location of the tumors but not their number. In particular, the localization of the tumor at the upper anterior or posterior bladder wall can be considered a limit due to a potential risk of peritoneal damage and the tumor location in bladder dome can be a challenging from a technical point of view (13).

This surgical technique, compared with traditional TUR, provides an intact tumor specimen containing detrusor muscle that allows pathologist to make accurate histopathological evaluation (17). In this type of specimen evaluation of circumferential and deep resection margins must be performed (16).

"En bloc" resection of bladder tumor surgical practice is not yet widely used while TUR remains the surgical procedure more used for non-muscle invasive bladder tumors and for large tumors that can be removed in fragments. The TUR specimens should be weighted in aggregate and processed completely, especially for TUR specimens up to 10 g. When papillary neoplasms are recognizable in these specimens, the number of tissue chips, that shows the lesion and gross tumor size should be recorded and, at least 1 cassette block per cm of tumor, up

to 10 blocks, should be sampled initially. For larger specimens, not entirely processed, additional blocks are recommended until complete embedding to rule out histological invasion of either the lamina propria or muscularis propria (4, 5). The European Association of Urology (EAU) guidelines recommend submitting exophytic part of tumor, the tumor base specimens, and the edges of the resection area in separate jars to simplify both the detection of muscularis propria, as a marker of complete local resection, and to evaluate the level of invasion (18).

Cystectomy Specimens

Standard radical cystectomy specimen includes the distal part of ureters, prostate, and seminal vesicles in men or urethra, adjacent vagina, and uterus in women. The organs adjacent to the bladder and the peritoneal lining allow to orientate the surgical specimen. Before dissection, it is recommended for cystectomy specimen an adequate fixation; this may be obtained either by distension of urinary bladder cavity with formalin injection (e.g., using a large gauge needle through the bladder dome or Foley catheter through the urethra) or by opening the bladder anteriorly from urethra to bladder dome before immersion in formalin (4). After adequate fixation, the orientated bladder specimen must be entirely and transversely sectioned at 5 mm intervals from bladder neck to dome, so that slices can be better compared with transverse tomographic scan or MRI (4, 8) (**Figure 1A**). The macroscopic description of the internal bladder surface should include the size, site, and appearance of tumor (papillary, solid, polypoid, or ulcerated) and the state of remaining mucosa. Moreover, the presence or absence of gross fat or serosa invasion should be recorded. When tumor is identified, the sampling should be adequate to its size (at least one section should be taken for each centimeter of tumor) and should allow to evaluate its deepest penetration on bladder wall, the grade, and the histological type. Sampling of normal appearing mucosa on different regions of the bladder wall should be made to detect occult multifocal carcinoma.

However, the tumor may not always be grossly visible, especially after re-TUR or pre surgical treatment, as a result of neoadjuvant therapy. In such cases, the sampling should be guided by prior surgical site, mucosal ulceration, or by cystoscopy or radiological images taken before tumor treatment. An extensive sampling is recommended in cystectomy with potential no residual tumor (4, 5, 8).

Whole mount technique can be used as an alternative to partial sampling by standard regular histological sections, even if whole mount method is not different to standard method in detecting adverse pathological features. Whole mount section advantages are a better view of bladder wall architecture and an easier comparison of the pathological findings with those obtained from radiological images (5) (**Figure 1B**).

PATHOLOGY REPORTING

The pathology report should include clinically relevant information as well as clinically useful gross and microscopic parameters. In this section, we consider the histological elements which should be present in the pathological report concerning both TUR/biopsy and cystectomy specimens. Currently, the International Collaboration on Cancer Reporting (ICCR) (19, 20) has elaborated a checklist for bladder cancer pathology report drafting considering dataset provided by several pathological anatomy organizations (http://www.iccr-cancer.org/datasets/published-datasets/urinary-male-genital/ut-biopsy-and-tr; and http://www.iccr-cancer.org/datasets/published-datasets/urinary-male-genital/bladder). In addition, ICCR has developed collaborations with other international cancer organizations responsible for neoplasm staging, such as American Joint Committee on Cancer (AJCC) and Union for international Cancer Control (UICC). The ICCR checklist includes the indications provided by the last WHO Classification of bladder tumor (21).

Histological Tumor Types

At present, different histological tumor types of bladder cancer are reported according to the 2016 WHO classification of urinary bladder tumors (21). The urothelial carcinoma is classified as such when there is any identifiable urothelial component, such as urothelial CIS. It is well-known that urothelial carcinoma may show unusual morphologic features that represent a divergent differentiation from 7 to 81% in various series (22–24). When urothelial carcinoma is not in pure form but shows divergent morphologies (**Figure 2A**), the histological tumor type retains the designation of urothelial carcinoma with associated histological subtype (e.g., squamous and glandular) and the percentage of each component of the tumor should be provided, because of its prognostic implication (22, 23, 25).

Neuroendocrine tumors (**Figure 2B**), such as small cell neuroendocrine carcinoma or large cell neuroendocrine carcinoma, are an exception to this rule, because regardless of the quantity of this component, it is recommended reporting all cases with a neuroendocrine carcinoma component as neuroendocrine tumor (19–21). These neuroendocrine tumors express immunohistochemical markers, such as synaptophysin (as shown inset in **Figure 2B**), chromogranin, and CD56 (21).

From a clinical point of view, the cases with a small cell neuroendocrine carcinoma component, are managed as small cell neuroendocrine carcinoma (26, 27). Few data exist about large cell neuroendocrine carcinomas, but they should probably be treated in the same way (28). Moreover, the ICCR suggested indicating neuroendocrine carcinoma component percentage because it influences carcinoma treatments, particularly the use of newest treatment, such as immunotherapy (19, 20).

WHO 2016 describes several variants of urothelial carcinoma and some of these have prognostic or therapeutic implications (21). These variants may represent a risk for bladder cancer under staging in the surgical specimens (29).

The nested type variant is a tumor with deceptively benign appearance that mimics von Brunn's nests and can be confused with von Brunn's nest hyperplasia if not invading the detrusor muscle. The tumor growth pattern varies from solid expansive to infiltrative nests without nuclear atypia that is observed most frequently in the deeper part of the tumor (30). Cytokeratins 20, 7, and p63 are expressed in nested type variant by immunohistochemistry (29, 31).

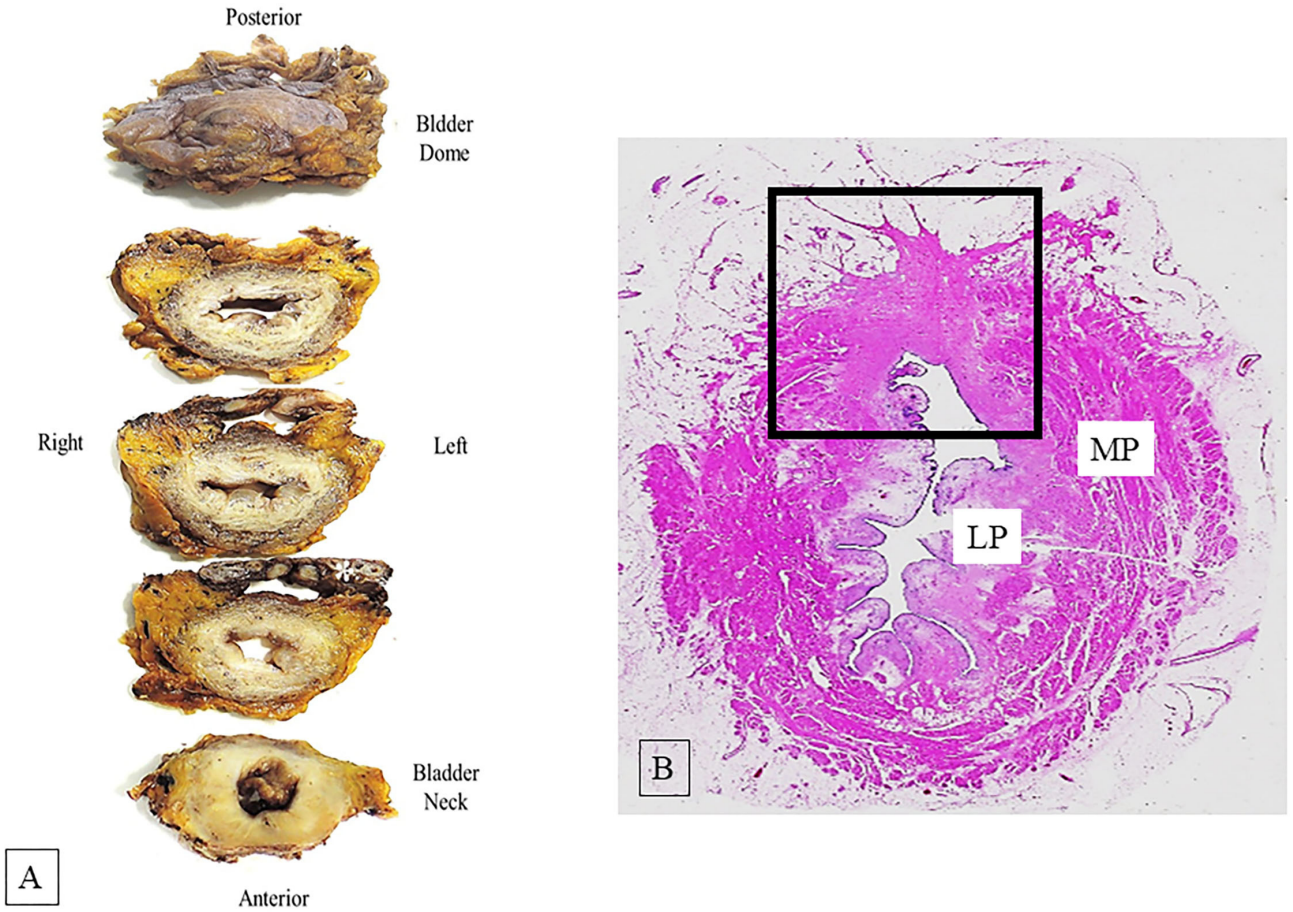

FIGURE 1 | (A) Serial dissected bladder transversely from the bladder neck to the dome at 5 mm intervals. The asterisk indicates the seminal vesicles. **(B)** Hematoxylin-eosin whole mount section shows bladder wall architecture; muscularis propria (MP) and lamina propria (LP). The square area indicates a site of a previous transurethral resection (TUR) showing fibrosis of the bladder wall.

A nested type tumor is considered a high-grade carcinoma and when it has been compared with urothelial carcinoma, it has displayed more frequent advanced tumor stage and increased rate of nodal metastasis (32, 33), this may be related to morphological features of this variant (similar to von Brunn's nests) delaying diagnosis of malignancy (34). In any case, patients with nested variant compared with those with pure urothelial carcinoma at the same stage have similar oncological outcome with no difference in recurrence rate and survival when treated surgically (35).

Plasmacytoid/diffuse variant is characterized by individual cells that look like plasma cells and single cells with cytoplasmic vacuoles can be present (**Figure 2C**). This tumor shows no extracellular mucin production (29, 36) and displays a diffusely infiltrative growth pattern with minimal stromal reaction, and frequent peritoneal carcinomatosis. Plasmacytoid carcinoma typically express urothelial markers, such as p63 and GATA3 and CD138, a plasma-cell marker (29, 31).

At presentation, plasmacytoid variant has a greater chance for higher-stage disease when compared with conventional urothelial carcinoma, showing metastasis and surgical margin positivity. The positivity of margins is due to both the capacity of tumor cells spreading in single file and lack of desmoplastic reaction, that makes difficult to determine the surgical plane between the tumor and normal tissue.

Identification of plasmacytoid variant is important to ensure an adequate resection at the time of cystectomy (37).

Therefore, diagnosis of plasmacytoid variant is important in the first TUR specimens, because an immediate cystectomy should be considered in early invasive tumors (i.e., pT1) (38, 39) while advanced disease appears to be chemotherapy responsive (29).

Lymphoepithelioma-like carcinoma (LELC) variant of urothelial carcinoma resembles the nasopharynx lymphoepithelioma, but unlike this, it is not related to Epstein-Barr virus. It is composed of nests, sheets, and cord of poorly differentiated cells with pleomorphic nuclei, prominent nucleoli, and indistinct cytoplasmic borders with syncytial appearance. A characteristic feature of this tumor is a dense infiltrate of lymphoid cells that may mask the carcinoma cells (40).

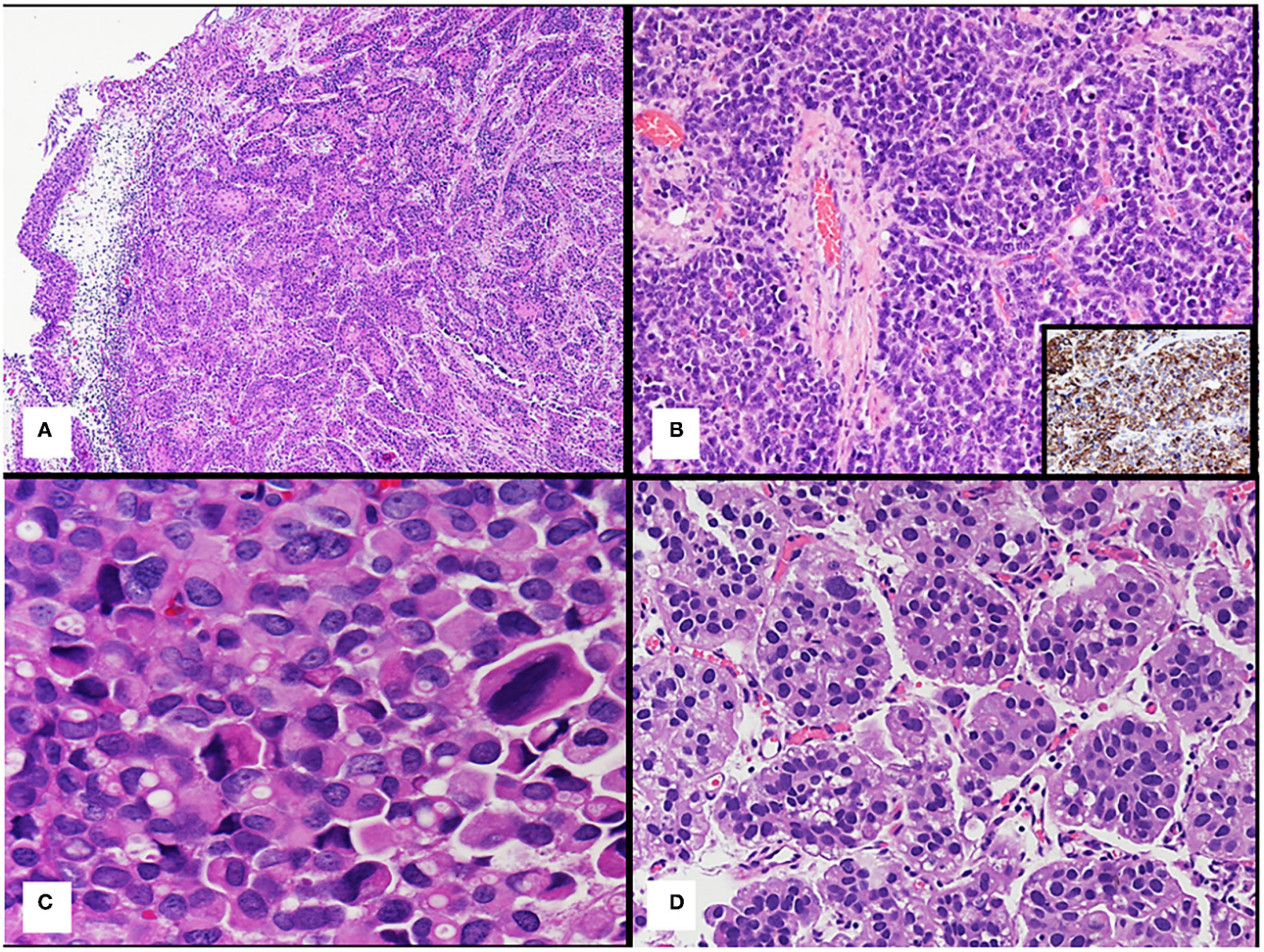

FIGURE 2 | (A) Urothelial carcinoma with squamous divergent differentiation. **(B)** Bladder neuroendocrine tumor; the inset shows positive immunohistochemistry staining for synaptophysin of the tumor. **(C)** Plasmacytoid urothelial carcinoma. **(D)** Micropapillary urothelial carcinoma.

Immunohistochemistry for cytokeratins (7 and 20) and urothelial markers (p63, GATA3, and uroplakin) highlights the epithelial cells for the diagnosis of LELC (29, 31).

Pure or predominant form of this variant appears to have a good prognosis with low metastatic potential (40) and a very favorable response to chemotherapy while mixed form LELC has a prognosis depending on the other variant present in the tumor. Therefore, cystectomy should be recommended in these last cases due to association with highest disease-free survival rate (8%) compared with TUR or partial cystectomy (41–43). A recent study showed that LELC tumors express PD-L1, this finding suggests to use immune checkpoint PD-L1 inhibitors as a therapeutic option (41).

Micropapillary urothelial carcinoma is characterized by small clusters of tumor cells without fibrovascular cores surrounded by empty spaces due to prominent retraction artifact that may mimic vascular invasion (44) (**Figure 2D**).

This variant shows positive stain for cytokeratin 7 and 20, epithelial membrane antigen (EMA) and Mucin 1 (MUC1) (29, 31).

A micropapillary tumor is an aggressive variant of urothelial carcinoma, at the time of detection more than 95% of these tumors are muscle invasive and in advanced stage, and lymph node involvement occur up to 35% of the patients (45, 46). This variant is frequently mixed with conventional urothelial carcinoma or other variant, and some studies suggest that any amount of micropapillary variant, even <10% is significant in urothelial carcinoma and should be reported (45, 47). In addition, some studies show contradictory results concerning micropapillary variant aggressiveness compared with pure urothelial carcinoma in the patients who underwent cystectomy (48).

In any case, non-muscle invasive micropapillary tumor is associated with high rate of progression to muscle invasive disease, and some studies have observed that this tumor

is unresponsive to intravesical therapy with BCG so early cystectomy is considered the standard management in most urological centers (29, 48, 49).

Considering muscle-invasive disease, protocols for neoadjuvant chemotherapy administration are not clear, so some authors indicated the immediate cystectomy while others recommend cystectomy with neoadjuvant chemotherapy (41, 50).

The molecular studies have shown that micropapillary carcinoma is characterized by HER2Neu overexpression and activation of miR-296 and RUVBL1 target genes showing relevant insights for future targeting therapy (47, 51).

Sarcomatoid urothelial carcinoma is an aggressive variant of urothelial carcinoma characterized by both epithelial and mesenchymal malignant differentiation, and undifferentiated high-grade spindle cell sarcoma is the mesenchymal component observed most frequently. Heterologous malignant elements may be present (e.g., osteosarcoma, chondrosarcoma, rhabdomyosarcoma, and leiomyosarcoma) (52, 53). In sarcomatoid urothelial carcinoma, the two components, carcinomatous and sarcomatous, are present in variable amount, but in the most cases sarcomatous component represents >50%. This variant may show prominent myxoid and sclerosing stroma, and that makes the diagnosis challenging.

This malignant neoplasm can be confused with spindle cell benign neoplasm or it can be under staging, especially in the TUR specimens, because spindled morphology of this neoplasia may obscure the muscularis (2, 29).

As previously described, sarcomatoid urothelial carcinoma is a biphasic tumor and immunohistochemical features evidence this aspect. The carcinomatous component is positive for the epithelial markers (i.e., AE1/AE3 and keratin CAM 5.2) and for EMA, as well as it is positive for mesenchymal marker as vimentin in ∼80–90% of tumors. In addition, the sarcomatous component is always positive for vimentin while it can express one or more epithelial markers. High molecular-weight cytokeratin is the marker most frequently expressed in the sarcomatous component. Moreover, immunohistochemical expression of urothelial differentiation markers, such as p63 and GATA3, although focal, can be useful for the diagnosis of this variant (21, 29, 31).

Sarcomatoid urothelial carcinoma frequently occurs at an advanced stage and it has a poor prognosis when compared with pure urothelial carcinoma (52, 53).

The survival for this type of carcinoma does not appear different in cases underwent to cystectomy compared with those receiving neoadjuvant or adjuvant chemotherapy (54).

Histological Tumor Grade

Histological tumor grade is a crucial parameter especially for non-invasive papillary urothelial tumor to guide the choice of therapy. The 2016 WHO (21) and more recently the ICCR (19, 20) recommend to use the same grade system adopted by WHO 2004 based on those initially proposed by the International Society of Urological Pathology (ISUP) in 1997 (55), whereas the use of other grading systems is considered as optional and it should be indicated.

The 2016 WHO classification system includes two categories of non-invasive bladder tumor, i.e., flat and papillary. The first is named urothelial CIS and the second type consist of papillary urothelial neoplasm of low malignant potential (PUNLMP) and papillary urothelial carcinoma.

A urothelial CIS is a flat non-invasive urothelial lesion of variable thickness, devoid of papillary structures containing cytologically malignant cells. It is very often multifocal and isolated ∼3% of cases. It is present with a synchronous non-muscle invasive urothelial carcinoma or with muscle invasive carcinoma in 50 and 60%, respectively (21, 56).

Papillary urothelial neoplasm of low malignant potential is considered a neoplasm unable to invade or metastasize, whereas papillary urothelial carcinoma is divided in two-tiered group: low and high-grade reflecting the different risk of progression to invasive carcinoma and death from bladder cancer (57). It is well-known that papillary urothelial carcinoma can present grade heterogeneity that has been reported in 3–43% of papillary urothelial lesions. Some studies indicated that mixed grade tumors should be labeled as high-grade tumors considering the percentage of high-grade components, but because of limited data, the cut-off utilized seem to be arbitrary (58–60). A recent study has demonstrated that low-grade areas in mixed grade papillary urothelial cancer showed molecular changes associated with disease progression (e.g., CDKN2A deletion) suggesting that molecular changes occur early and before morphological changes (61).

These findings support the current recommendation by the WHO 2016 and ICCR that the grade of the tumor depends on the highest-grade present in the lesion, so even if the lesion shows focal or minimal high-grade component, it has to be considered a high-grade tumor. In addition, the International Consultation of Urological Disease (ICUD) suggests that the percentage of the tumor high grade component should be recorded if it is <10% in the pathological report (62). Regarding invasive urothelial carcinoma, it should be considered as high grade (19, 62).

Extent of Invasion

Tumor invasion extension through the bladder wall is the criteria to assign the pathologic stage (pT) and at present, the 2017 version of AJCC Tumor-Nodes-Metastasis (TNM) classification is used (63, 64) (**Figure 3A**). Tumor staging can be difficult for pathologist so in this section, we will discuss the most common problems related to bladder cancer staging in different conditions of surgical specimen.

TUR or Biopsy

Pathologic Stage 1 (pT1)

Pathologic stage 1 (pT1) is defined by presence of tumor cells invading the sub-epithelial connective tissue (lamina propria), underneath the basement membrane, but not the muscularis propria (MP).

Some common diagnostic pitfalls are bound to surgical and excision factors, such as cautery injury, tangential section, or poor orientation of specimens. Other difficulty is due to the tissue reaction factors, such as desmoplastic stroma, inflammatory infiltrate, which may obscure single tumor cell infiltrating

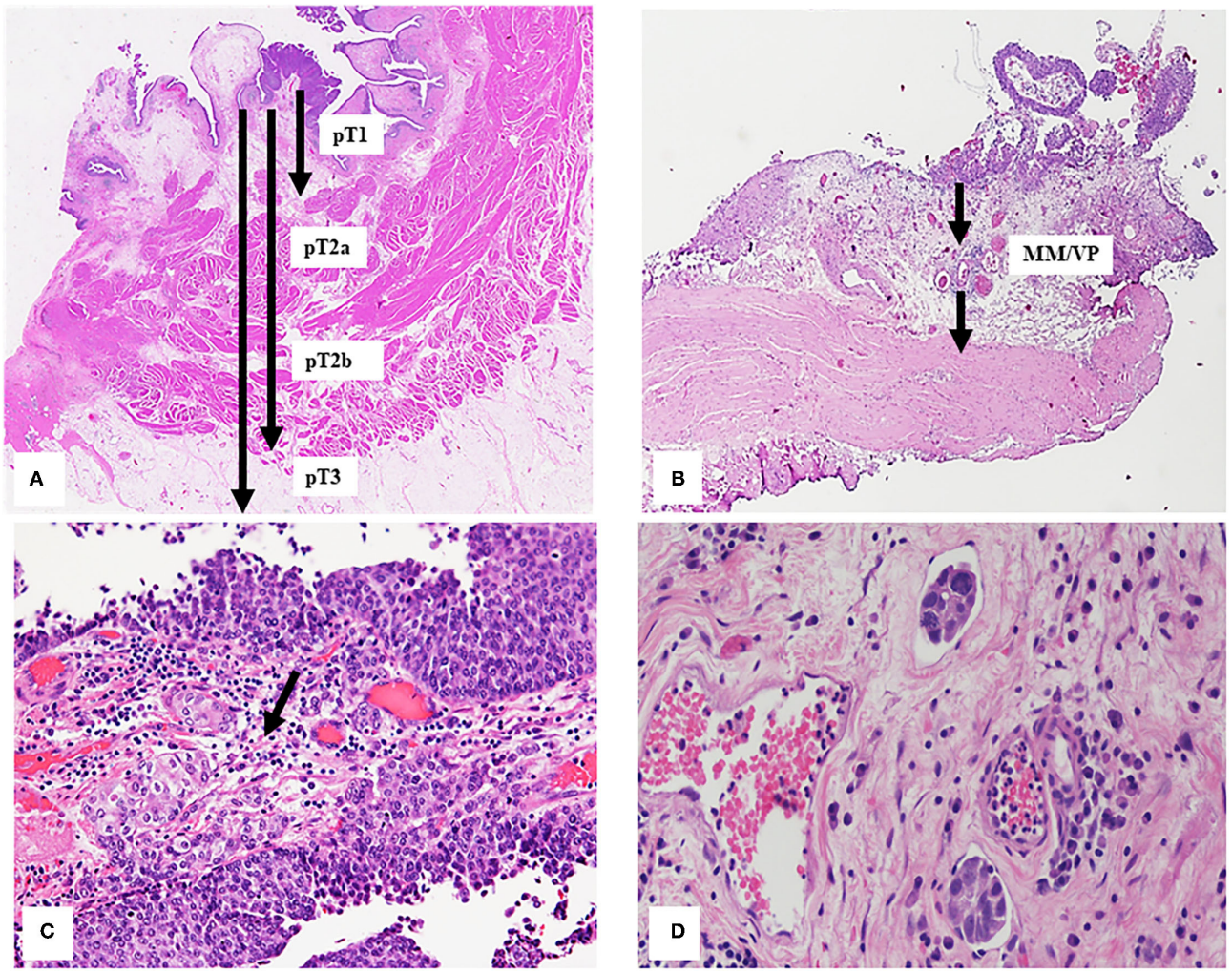

FIGURE 3 | (A) Extension of bladder tumor transmurally invasion classified considering 2017 version of AJCC TNM classification as described in the text. The arrows indicate different depths in relation to the different histological tumor stages. **(B)** Bladder wall Black arrows indicate the vascular plexus (VP) and the muscularis mucosae (MM), these divide the lamina propria in two part and represent the landmark to subclassified T1 tumors. **(C)** The arrow indicates the urothelial carcinoma infiltrating the lamina propria. **(D)** Small groups of tumor cells into two lymphatic vessels.

the lamina propria, or bladder epithelium and wall iatrogenic changes due to radiation therapy for pelvic cancers. In addition, tumor characteristics can make difficult the interpretation of the staging, such as some urothelial carcinoma variants (e.g., nested type or micropapillary) or CIS spread into von Brunn's nest (8, 65, 66) as well as the involvement of muscle fibers by invasive tumor. Several studies showed tumor up-staging (from 3 to 13%) or down-staging (from 15 to 56%) for pT1cases (67, 68) and also between expert genitourinary pathologists, a full agreement was reached in 47% of the pT1 cases (68) because the diagnosis of lamina propria invasion or the infiltration of the muscularis propria can be challenging (8, 66). In fact, the muscularis mucosae (MM) contained in the lamina propria can represent a confounding element to a correct diagnosis because the smooth muscles fibers constitute both MM and MP. In addition, the MM is not a complete layer in bladder and can be hyperplastic especially in the dome and the lamina propria is thinner in the bladder trigone and neck regions where, on the contrary, the MP is both thicker and more superficial (65, 66). Recently, smoothelin was proposed as a promising immunohistochemical marker to distinguish MP from MM which is usually weak or negative for smoothelin. It can be difficult to discriminate MM and MP in the TUR specimens when smoothelin staining is modest and without MP present as internal reference. Due to these limitations, the use of smoothelin is not currently recommended routinely (69). Other muscle markers, such as desmin and caldesmon were tested but unfortunately do not show unequal staining of MM and MP. If indecision between MM or MP involvement remains, this should be commented in the report (20). Last WHO Classification, CAP, ICUD, and ICCR

recommend substaging of pT1 but no indication is provided on methods to use for evaluating invasion extension. The anatomical or quantitative methods were proposed in literature to sub classify pT1 tumor, most of them correlate with recurrence free survival, progression free survival, and cancer specific survival. The anatomical method to sub classify pT1 tumor is based on the deep of invasion using MM or LP vascular plexus (its surrogate) as the histological landmark (**Figure 3B**). So, pT1 tumors can be classified in two or three sub classes (pT1a, b, or c) (70, 71). Recent meta-analysis showed that clinicians should treat patients with T1b/c substaging as having risk on a par with invasive bladder cancer (72).

The most frequently used quantitative methods to substage pT1 tumors is measuring the depth or maximum linear length of the invasive focus. The depth of invasive tumor is taken perpendicular to the mucosal surface while the maximum linear length of the invasive tumor can be the aggregated length of invasion foci; this last method is less affected by the orientation of the specimen (73–75) (**Figure 3C**). Over time, different cut-off points have been proposed for both methods to provide more information concerning risk of pT1 tumor recurrence and progression. Cheng et al. (76) have suggested a cut-off of 1.5 mm of depth of invasion calculated from basement membrane, others have suggested different aggregated invasive tumor lengths (73–75, 77, 78). In particular, Hu et al. (78) suggested 5 mm as cut-off of aggregated invasive tumor length for pT1 tumor recurrence, while Leivo et al. (74) proposed 2.3 mm as the optimal cut-off, which is larger than previously tested cut-offs of ∼0.5 and 1.0 mm measurements (73, 75) but smaller than more generous of 5 mm proposed by Hu et al. (78) for risk of pT1 tumor progression.

Regardless of the method used, an assessment of the depth and/ or extent of lamina propria invasion in pT1 cases should be provided in the pathological report.

Cystectomy

Pathologic Stage 0 (pT0)

Pathologic stage 0 (pT0) is assigned when residual tumor is not present in the cystectomy specimens after a previous cancer diagnosis in biopsy or TUR specimens or after neoadjuvant chemotherapy (yT0) (63, 64). The rate of pT0 is from 5 to 20% in contemporary cystectomy series without preoperative chemotherapy (79, 80) and comes up to 46% in cystectomy series after neoadjuvant chemotherapy (81).

Clinical indications about the site of the neoplasia should be considered when cystectomy specimens are evaluated for residual disease if no grossly apparent lesion exist or to find the site of previous TUR. In both cases, the suspicious area should be completely evaluated and if no residual cancer is found the case can be reported as pT0 (19).

Pathologic Stage 2 (pT2)

Pathologic stage 2 (pT2) bladder carcinoma is defined by tumor invasion into muscularis propria. pT2 bladder cancer is sub classified in two categories on the base of depth invasion of the muscularis propria: in pT2a the tumor invades the inner half (superficial part), while in pT2b the tumor invades the outer half (deep part) of the muscle wall (63, 64). Detrusor muscle anatomy does not always allow an easy distinction between inner and outer part of muscularis propria, and pathologist has to divide arbitrarily the muscle wall (65). These factors caused contradictory results in studies investigating oncological outcome of pT2 substaging in radical cystectomy (82–84).

Pathologic Stage 3 (pT3)

Pathologic stage (pT3) bladder carcinoma is defined by tumor invasion into peri-vesical soft tissue, such as peri-vesical fat. Fat invasion evaluation could seem easy but it can be challenging because the interpretation of microscopic peri-vesical tissue invasion could be subjective (19) as the junction between the outer layer of the muscularis propria and the peri-vesical fat is badly defined. The deeper part of muscularis propria shows haphazardly separated muscle bundles without clear demarcation with adipose peri-vesical tissue. Anatomical aspects and tumor related factors as dense fibrosis, desmoplasia, obscuring inflammation, and lympho-vascular invasion should be considered in the interpretation of tumor invasion beyond the muscularis propria (85). Invasive carcinoma surrounded by desmoplastic reaction, even if it does not touch the peri vesical fat but it is beyond the muscularis propria, should be considered pT3 as recommended by ICCR (19). pT3 bladder cancer is sub classified in two categories: pT3a (tumor with microscopic extravesical extension) and pT3b (tumor with gross extravesical extension). A tumor described as grossly involving the peri-vesical soft tissue requires histologic confirmation before it is considered in pT3b category (19).

Lymph Vascular Invasion

Lymph vascular invasion (LVI) is characterized by the presence of small group of tumor cells into lymphatic or blood vessels (**Figure 3D**). Its identification can be misleading in surgical specimens because retraction artifact around nest of invasive tumor cells (e.g., micropapillary variant) or peritumoral stroma retraction (86).

LVI detection can be difficult using hematoxylin and eosin-stained section, so immunohistochemistry technique (CD31, CD34, and D240) can be necessary but conflicting data exist on immunohistochemical staining for diagnosis of LVI in bladder cancer, so this technique should be used only in selected equivocal cases (1). Several studies suggested that LVI is an independent predictor of poor disease outcome both in TUR and cystectomy cases (1, 87). LVI presence should be indicated in the pathological report as the ICCR requested.

CONCLUSION

In conclusion, we can affirm that the handling and pathological evaluation of bladder cancer surgical specimens are crucial to

provide guidance for patient treatment. In this review, we have addressed the most discussed topics for the interpretation of surgical bladder samples requested from the major international pathology organizations that should be related in the pathological report. In particular, we considered the major issues that arises in the evaluation of both TUR and cystectomy samples to improve the collaboration between pathologists and urologists.

AUTHOR CONTRIBUTIONS

RMa contributed to the conception of the manuscript. RMa and DM contributed to the writing of the manuscript. GT, LP, and RMo contributed to the critical revision and final approval of the article. All authors contributed to the article and approved the submitted version.

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frontiers in Surgery

REVIEW
published: 21 February 2022
doi: 10.3389/fsurg.2022.762027

Role of Macroscopic Image Enhancement in Diagnosis of Non-Muscle-Invasive Bladder Cancer: An Analytical Review

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Edited by:
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Specialty section:
This article was submitted to Genitourinary Surgery, a section of the journal Frontiers in Surgery

Received: 20 August 2021
Accepted: 17 January 2022
Published: 21 February 2022

Citation:
Mulawkar PM, Sharma G, Tamhankar A, Shah U and Raheem R (2022) Role of Macroscopic Image Enhancement in Diagnosis of Non-Muscle-Invasive Bladder Cancer: An Analytical Review. Front. Surg. 9:762027. doi: 10.3389/fsurg.2022.762027

Early diagnosis of non-muscle-invasive bladder cancer (NMIBC) is of paramount importance to prevent morbidity and mortality due to bladder cancer. Although white light imaging (WLI) cystoscopy has long been considered the gold standard in the diagnosis of bladder cancer, it can miss lesions in a substantial percentage of patients and is very likely to miss carcinoma *in situ* and dysplasia. Tumor margin detection by WLI can be inaccurate. Moreover, WLI could, sometimes, be inadequate in distinguishing inflammation and malignancy. To improve the diagnostic efficacy of cystoscopy, various optical image enhancement modalities have been studied. These image enhancement modalities have been classified as macroscopic, microscopic, or molecular. Photodynamic diagnosis (PDD), narrow band imaging (NBI), and Storz image 1 S enhancement (formerly known as SPIES) are macroscopic image enhancement modalities. A relevant search was performed for literature describing macroscopic image enhancement modalities like PDD, NBI, and image 1 S enhancement. The advantages, limitations, and usefulness of each of these in the diagnosis of bladder cancer were studied. Photodynamic diagnosis requires intravesical instillation of a photosensitizing agent and a special blue light cystoscope system. PDD has been shown to be more sensitive than WLI in the detection of bladder cancer. It is superior to WLI in the detection of flat lesions. Bladder tumor resection (TURBT) by PDD results in more complete resection and reduced recurrence rates. PDD-guided TURBT may have some role in reducing the risk of progression. Narrow band imaging provides increased contrast between normal and abnormal tissues based on neovascularization, thereby augmenting WLI. NBI requires a special light source. There is no need for intravesical contrast instillation. NBI is superior to WLI in the detection of bladder cancer. The addition of NBI to WLI improves the detection of flat lesions like carcinoma *in situ*. NBI is not useful in predicting invasive tumors or grades of tumors. NBI-directed TURBT reduces recurrence rates and recurrence free survival. But its efficacy in retarding progression is unproven. Image 1 S-enhancement utilizes software-based image enhancement modes without the need for a special light source or intravesical contrast instillation. This system provides

high-quality images and identifies additional abnormal-looking areas. Another advantage of this system is simultaneous side-by-side visualization of WLI and enhanced image, providing WLI images as the control for comparison. As with PDD, S-enhancement produces a lower rate of a missed bladder cancer diagnosis. The system significantly improves the diagnosis of NMIBC. The sensitivity and negative predictive value of image 1 S enhancement increase with the increase in cancer grade. A negative test by S-enhancement effectively rules out NMIBC. All the image enhancement modalities have proven their utility in improving detection and short-term cancer control. But none of these modalities have proven their utility in delaying progression, or in long-term cancer control. Cancer progression and long-term control are governed by the biological nature of cancer cells. Early detection by optical enhancement may not be of utility in this regard. Well-designed studies are needed to establish the efficacy of these modalities in the evaluation of patients with bladder cancer. The last word, in this regard, is yet to be written.

Keywords: bladder cancer, image enhancement, fluorescence cystoscopy, narrow band imaging, image 1 S

INTRODUCTION

Among urological cancers, non-muscle-invasive bladder cancer (NMIBC) has a better prognosis. But bladder cancer, as a whole, is the costliest solid tumor to treat (1). Although cytologic analysis, biomarkers, and imaging are useful tests, the mainstay of diagnosis rests on cystoscopy (1). White light imaging (WLI) cystoscopy, the most commonly utilized diagnostic modality (2), has been at the forefront of the diagnosis of bladder cancer for more than a century. WLI is the "Gold standard" for the diagnosis of bladder cancer (1, 3). Once detected, a lesion is managed by transurethral resection (TURBT) to know its histologic grade and stage. Around three quarters of patients are diagnosed by WLI and resection (2). Conventional cystoscopy can miss around 25% of bladder tumors (4). The technique of TURBT can be piecemeal or *en bloc,* and there is an important concern that new enhanced optical techniques can affect the *en bloc* resection of bladder tumors (5). However, the technique of resection during TURBT is not being discussed in this review.

The quality and adequacy of resection vary among institutions and from surgeon to surgeon. The risk of recurrence cannot be explained by the biology of a tumor alone (6). Possible mechanisms of recurrence are incomplete resection of tumors (overlooked residual tumors), microscopic residual tumors (regrowth of tumors), implantation of tumor cells in raw sites, and new occurrence of tumors (7). At least two of these mechanisms direct toward incomplete TURBT (8). Incomplete TURBT, especially in high-grade tumors, will negatively affect the prognosis of bladder cancer. Cystoscopy can also miss occult neoplastic changes in flat urothelial. Lesions in this category would be dysplasia, low- and high-grade intraurothelial neoplasia, and carcinoma *in situ* (CIS) (9). It is a well-known fact that WLI can miss some lesions. Such missed lesions can show up as "recurrences" on check cystoscopies. It is not technically possible to perform a perfect "text book TURBT" like complete macroscopic clearance, thorough resection of a tumor base, and separate resection of tumor margins. An incomplete TURBT, even if inadvertent, may change the prognosis of illness in a given patient. The risk of residual tumors is acceptably low at around 10% in Ta disease, but in T1 disease it could be as high as 62% (7). This residual tumor, labeled as recurrence in T1, is supposed to be a poor prognostic factor.

If not the biology, then other factors like a machine or humans behind the machine might be a factor influencing the completeness of TURBT. In this review, we talk about the machine. To improve the diagnosis of suspicious bladder lesions, optical enhancement modalities are used. This analytical review is about the role of macroscopic image enhancement modalities in the management of NMIBC.

MATERIALS AND METHODS

A PubMed search was performed with the following criteria: (bladder cancer) and (narrow band imaging); (Bladder cancer) and ("Fluorescence Cystoscopy"); (bladder cancer) and (SPIES); (bladder cancer) and (image 1S); (bladder cancer) and (optical enhancements). Initially, titles were screened to identify eligible articles followed by the screening of abstracts. Finally, full-text articles were read. In addition, individual searches were performed on Google Scholar, Scopus, ScienceDirect, and SpringerLink. Reference lists of the selected articles were also searched, and additional studies were included. All authors participated in the formulation of the search strategy and selection of the articles. There were no specific exclusion criteria. Editorials, reviews, opinions, debates, and letters to editors were also included if found relevant. There was no particular time frame applied for the literature. The advantages, limitations, and usefulness of different image enhancement modalities in the diagnosis and management of bladder cancer were studied. A total of 387 articles were retrieved; of which 127 were included and 260 were rejected. Most of the literature was combined in a simple narrative fashion.

RESULTS AND DISCUSSION

Classifications of Image Enhancement Technologies

Image enhancement technologies are usually classified as macroscopic or microscopic.

Macroscopic Techniques

Macroscopic technologies are also called "wide field view." In this, a surgeon diagnoses a lesion looking at mucosal morphology, color, and vessels. Macroscopic imaging identifies ulcers, erosions, papillary lesions, reddish or white spots, and the presence of vessels in the mucosa and neo-angiogenesis (10). Macroscopic techniques are further classified as wide-field WLI or contrast enhancement techniques.

Wide-Field WLI

High-definition camera systems (1,080 p) provide a >5-fold increase in image resolution. In addition, image filtering and zooming improve picture quality by more than 30% (11). Camara systems with a resolution higher than high definition are being used nowadays. Newer systems with 4 and 8K (UHD) are being used nowadays. Wide-field endoscopy also uses enhancement modalities like close focus, dual focus, optical zoom, and electronic zoom to gain better vision and have a low rate of missed lesions (10). Data on the impact of wide-field WLI on improved diagnosis and better outcomes in bladder cancer is awaited. Wide-field WLI, as a technique, is not part of this review.

Contrast Enhancement Techniques

Virtual Chromoendoscopy. Virtual chromoendoscopy improves the detection of lesions by changing spatial variance and scattering properties. Herein, either incident light is changed to selected waveforms or images generated after illumination are processed to transform color or change color tone, or change the contrast. Based on the stage of processing of light, VCE is further classified into three categories: pre-processing, post-processing, pre- and post-processing (10).

Commonly used pre-processing VCE techniques are narrow-band imaging (NBI), red dichromatic imaging, and blue light imaging. In pre-processing VCE, a modified spectrum of light is switched on, and it results in color transformation depending on the peak absorption of hemoglobin and different tissue elements. In post-processing VCE, incident light is white light, but processing occurs at the camera processor level by color transformation and color tone change to produce an effect similar to that of pre-processing VCE. Commonly used post-processing VCE techniques are Fujinon intelligent chromoendoscopy (FICE) and image 1 S (previously called Storz professional image enhancement system or SPIES). FICE is commonly used in gastroenterology but is not discussed further.

Fluorescence Endoscopy. This technique utilizes different fluorophores. A fluorophore is a fluorescent chemical compound that, on excitation with light, reemits light. Fluorescence signals emitted by fluorophores are dependent on wavelength. There are special optical fibers used in this system that block the false excitation of light and capture only a part of the emitted light, thereby producing a final image (10). Commonly used fluorescence endoscopy techniques are autofluorescence imaging (AFI), near-infrared imaging (NIR) with indocyanine green (ICG), and photodynamic diagnosis (PDD). AFI is commonly performed in gastroenterology. Urological applications of NIR and ICG include tumor localization, selective arterial clamping during partial nephrectomy, mapping of lymph nodes during radical prostatectomy, radical cystectomy and penile cancer, urethral viability assessment during urethroplasty and varicocelectomy (12, 13). NIR and ICG are not being discussed further.

Microscopic Techniques

These techniques visualize microstructural details of tissues and act like an optical biopsy, which acts like *in vivo* tissue biopsy. Confocal laser endomicroscopy (CLE) is a promising tool for the optical diagnosis of bladder cancer. Microscopic techniques are not part of this review.

Fluorescence Cystoscopy/PDD

Principle

The basic principle behind PDD is the selective emission of fluorescence by cancer cells. Commonly used substances are 5-aminolevulinic acid (ALA) and hexaminolevulinate (HAL). When instilled in the bladder, these are preferentially taken up by cells with high metabolic turnover. Drugs are incorporated in cellular hem biosynthesis metabolism, wherein prodrugs are converted to protoporphyrin IX (PpIX). Pathological and normal tissues have different concentrations of fluorescence molecules. These molecules are excited by light of an appropriate wavelength. After molecules relax to the ground state, they emit photons. Excitation photons have more energy than fluorescence photons. If the energy of light is more, the wavelength is short and vice versa. Hence, the light that is emitted from a fluorescence photon will have a longer wavelength than the illuminating photon. Hence, it is possible to discriminate between two types of light by PDD (14).

Technical Consideration

Equipment

A high-performance light source called Karl Storz D-Light C is used for PDD (15). The light source has a band pass filter, which produces blue light of 360–450-nm wavelength. This light source can be switched from white light to fluorescence mode with a footswitch. Rigid telescopes with 0-, 12-, 30-, and 70-degree vision are commonly used. Flexible cystoscopes and chip-on-tip video cystoscopes are also available (15, 16). Apart from Karl Storz, Germany, Richard Wolf, Germany also makes PDD systems (17).

Photosensitizing Agents

Typically, 5-ALA is used as a freshly prepared solution of 1.5 gm 5-aminolevulinic acid dissolved in 50 ml 5.7% sodium monohydrogenphosphate. Dwell time of 2–3 h is recommended (18). ALA yields high sensitivity especially for the diagnosis of CIS and pinpoint lesions. But the amount of ALA entering cancerous cells is limited, as the cellular uptake of ALA is

limited due to ionic structure. To overcome this shortcoming, an increased dose or increased dwell time is needed (19).

In contrast, the ester form of a drug penetrates cells better. Once inside a cell, the ester form is hydrolyzed back to ALA by tissue esterases. Lange et al. (19) are credited for publishing initial experiences in using 5-aminolaevulinic acid hexylester hydrochloride (HAL)-induced PDD. Advantages of HAL are better solubility in urine and water, higher local bioavailability, more PpIX formation at lower doses, homogenous fluorescence, less vulnerability to photobleaching, and excellent florescence intensity (20). Currently, HAL is available as Cysview (Photocure US, Princeton, NJ, United States) or Hexvix (Photocure ASA, 0275 Oslo, Norway) as a kit containing 100 mg hexaminolevulinate HCL powder and 50 ml phosphate-buffered saline as a diluent. It can be stored for 2 h at 2–8°C. The solution is usually prepared once a patient is admitted. Once the patient is ready, a catheter is passed. All urine in the bladder is emptied, and a freshly prepared HAL solution is instilled slowly in the bladder. The usual dwell time is 1–3 h. HAL should not be used with silver-coated catheters (15).

Intravesical HAL is usually well-tolerated. Common but not clinically significant adverse events reported are bladder spasm, dysuria, and hematuria (15). HAL is contraindicated in patients with a history of known allergy to HAL or a history of porphyria. There has been one case report of anaphylaxis after HAL or PDD (21).

Hexaminolevulinate can be used by the intravesical instillation route only. 5-ALA can also be used by intravesical and oral routes. A comparative randomized multicentre phase II/III study on oral 5-ALA in doses of 10 and 20 mg/kg was reported by Inoue et al. (22). The authors reported a higher rate of tumor detection with the 20 mg/kg dose and suggested this dose for clinical use (22). Nakai reported the diagnostic efficacy, safety, and tolerability of PDD with oral 5-ALA in a multicentre phase III trial. The dose used was 20 mg/kg of oral 5-ALA (23).

Hypericin, a plant derivative, is another fluorophore used in urology. Decreased susceptibility to photobleaching and better specificity are possible advantages of hypericin. But low solubility is an issue. Efforts to improve its solubility using solvents like polyvinylpyrrolidone and albumin are being investigated. Hypericin is well-tolerated, and its fluorescence is maintained up to 16 h (24). In a study to determine the optimum dose of hypericin, 225 ug for 30 min was concluded to be the optimum dose for intravesical instillation (25). In addition, pirarubicin has also been used for PDD (26).

PDD Cystoscopy Procedure

After a drug dwell time of 1 h, a patient is taken to an operating room. Rigid cystoscopy is performed. Initially, systematic WLI is performed, and lesions are marked in bladder map proforma. Then, the blue light is switched on, Initially, the scope is kept at the bladder neck to identify tangential artifacts. This ensures that the photosensitizing agent is applied properly (15). For a detailed evaluation of a lesion, the scope should be close and as perpendicular as possible to the bladder wall or a tangential artifact may occur. As the observation angle becomes more tangent, the bladder mucosa appears more fluorescent (27).

Lesions are seen as bright red fluorescence. The dark blue background illumination in PDD hampers proper depth perception. Hence, a biopsy is usually performed with white light. Similarly, resection is also usually performed with white light. Urine fluoresces green under PDD. Therefore, it is advisable to empty it periodically (15). It is advisable to perform a mapping cystoscopy initially before starting the resection as fluorescence fades with time.

The Learning Curve of PDD

Gravas et al. evaluated the learning curve of PDD with HAL (28). The authors compared the agreement statistics among cystoscopies performed by senior residents and experienced urologists. After an experience of 20 cases, inexperienced observers had good agreement with the experienced ones, which improved to an excellent agreement after 30 cases, suggesting a learning curve of around 20–30 cases (28). The experience of a urologist performing PDD is an important factor for the quality of PDD (29). An experienced urologist who takes more biopsies is likely to yield more false positives and resect more tumors. As the learning curve of urologists goes beyond 12–18 months, the number of false positives goes down (29).

Performance of PDD: Diagnosis, Residual Tumors, Short-Term Recurrence, and Effects on Treatment

Zaak et al. are credited for one of the first few clinical data on the role of PDD with ALA in bladder cancer. The authors found that PDD was more efficient than WLI and urine cytology in diagnosing high-risk urothelial lesions (18). In initial studies, PDD was performed using 5-ALA and HAL. Both the fluorescence agents have shown improved sensitivity but poor specificity (**Table 1**).

Hexaminolevulinate is the most commonly used agent in PDD, and literature regarding HAL is reviewed preferentially. Grossman et al. reported their experiences in a multicentric trial on PDD using HAL (35). The authors found that PDD was efficacious by detecting at least one more Ta lesion than WLI in 29% of patients and one more T1 lesion in 15% of patients. WLI detected at least one more Ta lesion than PDD in 9% of patients and one more T1 lesion in 5% of patients (35). Mean detection rates for PDD and WLI were 95 and 83% for Ta ($p = 0.001$) and 95 and 86% for T1 lesions, respectively. Interestingly, all additional lesions detected by PDD in this study were grade 3.

Stenzl et al. published a prospective randomized trial studying the effects of improved detection of bladder cancer on early recurrence rates (36). The authors recruited patients with an increased risk of recurrences like more than one initial or recurrent tumor and recurrence within 1 year of previous bladder cancer. These patients were randomized to TURBT with WLI or PDD using HAL. Recurrence rates were 56% in WLI and 47% in the PDD groups ($p = 0.026$). There was a 16% relative reduction in the risk of recurrence (36).

Geavlete et al. reported recurrence rates of 23.2% with WLI and 5.3% with PDD in a HAL group in over 18 weeks follow-up (37). In another study, the same group reported their experiences in a single institute prospective randomized trial comparing TURBT with WLI or HAL PDD (38). Tumor detection rates were

TABLE 1 | Characteristics of sensitivity and specificity of photodynamic diagnosis (PDD) and white light imaging (WLI).

References	Photosensitiser	PDD sensitivity	PDD specificity	WLI sensitivity	WLI specificity
Kriegmair et al. (30)	5ALA	97	67	73	69
Riedl et al. (4)	5ALA	95	43	76	*
Filbeck et al. (31)	5ALA	96	67	68	66
Koenig et al. (32)	5ALA	87	59	69	89
Jichlinski et al. (33)	HAL	96	43	73	43
Schmidbauer et al. (34)	HAL	97	*	78	*

No data.

better in the PDD arm. Three-month recurrence rates were 7.2 vs. 15.8% in PDD vs. WLI arm. One-year recurrence rates were 21.6 vs. 32.5% respectively. Two-year recurrence rates were 31.2 and 45.6% (38). Fradet et al. published results of phase III multicentre trial wherein WLI was compared with PDD using HAL (39). This study was mainly directed toward the detection of CIS. Fifty-eight out of a total of 196 patients had CIS lesions. A total of 113 lesions were detected in these 58 patients. Of these, 92% of the lesions were detected by PDD and 68% by WLI, but 5 (of 113) CIS lesions were detected only by random biopsy of normal-looking mucosa. Although tumor detection rates were higher in the PDD group, there were some, albeit scant, numbers of lesions detected only by WLI.

Schmidbauer et al. reported a within-patient comparison of WLI and PDD with HAL (34). The study was designed to evaluate the detection of CIS. In this study, 83 out of 211 patients had CIS. Among these, 22% were detected by PDD alone, 75% of CIS lesions were detected by WLI as well as PDD, and 2% of lesions were detected by WLI alone and 1% by non-guided biopsy. A lesion detected by non-guided biopsy was located in the prostatic urethra where PDD is less discriminating because of tangential artifact (34). PDD cystoscopy detected a significantly higher number of additional CIS lesions than WLI. Interestingly, in this study, false positive rates for PDD were similar to those for WLI cystoscopy (13 vs. 10%). PDD cystoscopy detected 28% more patients with CIS lesions than WLI cystoscopy (34). Blanco et al. evaluated the performance of HAL PDD in the detection of urothelial lesions in patients with a high risk of progression (40). The study also included patients who have received BCG. HAL PDD had 90.1% sensitivity and 87.5% specificity. The positive predictive value was 95.2%, and the negative predictive value was 77.8% (40).

There are some doubts on whether additional detection of few more papillary tumors has any impact on treatment planning. Filbeck et al. reported a within-patient evaluation to see if the addition of PDD with 5ALA during TURBT would lead to therapeutic consequences (41). In this study, the addition of PDD led to the detection of additional tumors missed by WLI in 5.1% of patients, and the gain of additional information in 15.3% of patients leading to a change in treatment strategy in 9% of the patients. Jocham et al. evaluated whether the improvement in detection by PDD had an impact on treatment planning (42). This was an open, comparative, within-patient study. Anonymized data from patients were sent to a blinded urologist, and he was asked to formulate a treatment plan as per European urology association bladder cancer guidelines. The diagnosis with PDD led to the recommendation of additional treatments like BCG and topical or prophylactic chemotherapy, TURBT, and cystectomy. In two patients where cystectomy was advised after PDD imaging, no tumor was detected on WLI. TURBT with PDD resulted in improved treatment decisions in a significant number of patients ($p < 0.0001$) (42).

The role of HAL PDD in the screening of men aged 60–70 years with microscopic hematuria and positive bladder tumor marker was evaluated by Hedelin et al. (43). The authors reported that HAL PDD in this screening scenario was not useful. Rather, they suggested HAL PDD cystoscopy in older male smokers with >25 RBC/ul (43).

A prospective phase II study on PDD using a flexible cystoscope and rigid cystoscope for diagnosis of bladder tumors found PDD with flexible cystoscope to be slightly inferior to PDD with rigid cystoscope (44). On the contrary, in a recent study, Drejer and colleagues highlighted the beneficial effects of flexible cystoscopy in post TURBT follow up. A reduction by 33% was noted in the short term recurrence rates over a median follow-up of eight months (45).

Mowatt et al. from Aberdeen Technology Assessment Review Group reported a meta-analysis of 27 studies including 2,949 patients (46). In patient-level analysis, PDD yielded 92% sensitivity (95% CI 80–100%); on the contrary, WLI had 71% sensitivity (95% CI 49–93%). But the specificity of PDD was lower at 57% (95% CI 36–79%), whereas the specificity of WLI was 72% (95% CI 47–96%). In biopsy-level analysis, PDD had a sensitivity of 93% (95% CI 90–96%); WLI had a sensitivity of 65% (95% CI 55–74%). The specificity of PDD was 60% (95% CI 49–71%). The specificity of WLI was 81% (95% CI 73–90%). Considering 5-ALA and HAL separately (46) in patient-based detection, the median sensitivity and specificity of 5-ALA were 96% (64–100%) and 52% (33–67%), respectively; whereas for HAL sensitivity was 90% (53–96%) and specificity was 81% (43–100%). For biopsy-based detection, sensitivity and specificity were 95% (87–98%) and 57% (32–67%) for 5-ALA, and 85% (76–94%) and 80% (58–100%) for HAL. Thus, the sensitivity of PDD was higher, but specificity was lower than that of WLI. For detection of lower risk and less aggressive tumors, the sensitivity of PDD was comparable to that of WLI in patient-level detection but higher in biopsy-level detection. For detection of high-risk and more aggressive tumors including CIS, the sensitivity of PDD

was better in patient-level as well as biopsy-level detection. In this meta-analysis, PDD was found to be more sensitive but less specific than WLI both at the patient level and biopsy level. This advantage of PDD was more pronounced in the detection of more aggressive and high-grade tumors including CIS.

Mowatt et al. reported a meta-analysis comparing WLI with PDD (46). In post TURBT repeat cystoscopies done between 10–14 days and 10–15 weeks, the overall (both WLI and PDD combined) relative risk of pTa and pT1 residual tumors was 0.37 (95% CI 0.20–0.69). Residual tumors in the WLI group were RR 0.32 (95% CI 0.15–0.7), and in the PDD group, RR was 0.26 (95% CI 0.12–0.57). The PDD-guided TURBT was beneficial in terms of residual tumor rates. In the Mowatt meta-analysis (46), two studies reported outcomes of recurrence-free survival. The benefit of recurrence-free survival in favor of PDD was observed at 24 months but not at 12 months.

However, the beneficial effects of PDD in recurrence are not universal across all studies. A Swedish multicenter prospective randomized study on PDD using 5-ALA compared with WLI was reported by Schumacher (47). Patients were randomized to TURBT with WLI and TURBT with PDD. All follow-up cystoscopies were performed under WLI. The authors did not find any advantage of PDD over WLI in the form of recurrence-free and progression-free survival rates. However, more lesions were detected in the PDD group on the within-patient comparison. The results of this study have been criticized for inexperienced observers and mean exposure time of 5-ALA of only 2.1 h (48). A similar multicenter prospective placebo-controlled randomized trial was reported by Stenz et al. (49). In this trial, the tumor detection rate was higher in the PDD group than in the placebo group. However, the recurrence-free and progression-free survival rates were similar in the PDD and placebo groups.

A systematic review and meta-analysis by Shen et al. assessed the diagnostic accuracy and therapeutic efficacy of TURBT with WLI or PDD (50). In eight studies the comparator was 5-ALA, in 3 studies HAL, and 2 studies HAL and 5ALA; in one study, no record of fluorescence agent was available. Unexpectedly, this meta-analysis did not find PDD to be superior to WLI in tumor detection and CIS detection rates. Residual tumor rates were, however, higher in the WLI group. Short-term recurrence-free survival and progression-free survival were also not different in the WLI and PDD groups (50).

Burger et al. reported a meta-analysis containing raw data from prospective studies on 1,345 patients (51). WLI and PDD HAL were the comparators. PDD detected significantly more Ta [14.7%; $p < 0.001$; odds ratio (OR): 4.898; 95% CI, 1.937–12.39] tumors as well as CIS (40.8%; $p < 0.001$; OR: 12.372; 95% CI, 6.343–24.133) lesions than WLI. By PDD, at least one additional Ta or T1 lesion was detected in a quarter of the patients. Similarly, in a quarter of the patients, CIS was detected only by PDD. This improvement in detection was seen in primary recurrent cancers as well as patients with high and intermediate risks. One-year recurrence rates were lower in PDD than in WLI [34.5 vs. 45.4%, $p = 0.006$; RR 0.761 (0.627–0.924)]. This benefit was seen in Ta, T1, and CIS lesions and high-risk and low-risk groups (51). Similar outcomes were reported in a meta-analysis reported by Kausch et al. (52).

The Issue of False Positives, Post BCG PDD, and Other Limitations of PDD

One of the disadvantages of PDD is high false positive rates (1–26%) (53). Common causes of false positives during PDD cystoscopy are erythema, bladder inflammation, trauma due to cystoscope, prior intravesical therapy, squamous metaplasia, and scar from old resection site. Reasons for variable specificities of PDD across different studies are different dwell times of photosensitizing agent, the experience of urologist, differences in techniques and equipment, and differences in patient population and pathologic classification system used (27). With regard to false positives, HAL performs better than 5-ALA (46).

Photodynamic diagnosis is usually avoided within 3 months of intravesical BCG therapy because more than one BCG instillation during this period has a significant effect on false positive rates of PDD (54). Grimbergen et al. evaluated the effect of previous intravesical therapy (BCG, mitomycin, or epirubicin) on false positive rates (27). Patients were recruited in three groups; intravesical therapy within the prior 6 months, more than 6 months, and no intravesical therapy. False positive rates were 39.6, 30.6, and 25.7% ($p < 0.025$) (27). In this study, around one-third of lesions were overlooked by WLI, and one-third of these lesions were high-risk lesions. These high-risk lesions are likely to affect patient outcomes significantly. Therefore, the authors have recommended PDD shortly even after intravesical therapy (27).

Resection of all visible fluorescence mucosae yields better progression-free survival. It is possible that these false positive lesions may be premalignant. The role of p53 and p16 immunoreactivity in PDD false positive lesions, thinking these to be premalignant, was evaluated by Hendricksen et al., but little evidence for these lesions being premalignant was found (55). Matsuyama et al. determined genetic instabilities in the bladder mucosa suspected to be having CIS by 5-ALA-based cystoscopy (56). They found substantial early genetic changes in chromosome 9 in non-malignant fluorescence cells (56). As most of the studies did not perform random biopsies, the false negatives with PDD should be interpreted with caution. But most so-called false negatives are detected by WLI and should not affect the patient outcome (53). The optimum dwell time for HAL is 60 min. Patients with urgency may not retain the drug that long and may lead to reduced uptake. Improper maintenance of equipment, such as mixing the PDD equipment with other cystoscopy equipment, may be a source of error. Doing TURBT under blue light is technically challenging. Therefore, most surgeons do it with WLI. White light causes rapid elimination of PpIX, leading to photobleaching. This may lead to missed tumors during TURBT especially if it is prolonged. Apart from these, the limitations of PDD are minimal (53).

Long-Term Effects: Effects on Long-Term Recurrence and Progression, and Effect on Post-radical Cystectomy Outcome

Stenzl et al., in their multicentric prospective randomized trial on cystoscopy and TURBT with WLI and PDD using HAL,

reported improved detection, but a subsequent decrease in disease progression was not found (36). In the meta-analysis of Mowatt et al. (46), the benefits of using PDD TURBT favored PDD but were not statistically significant in the long run. The along-term study showed trends toward lower cystectomy rates in patients who underwent PDD TURBT (48).

Babjuk et al. from the Czech Republic reported their data-randomized trial studying long-term follow-up of patients undergoing TURBT with WLI or PDD. The mean follow-up in WLI was 20.7 months and 22.4 in the PDD group. At 1 and 2 years, recurrence-free survival was 39 and 28% in the WLI group, whereas it was 66 and 40%, respectively, in the PDD group ($p = 0.008$) (8). The beneficial effect of TURBT with PDD was significantly higher on multiple and recurrent tumors. Similarly, the beneficial effect was more evident at 1 year than 2 years (8). Another set of long-term follow-up data was presented by Daniltchenko et al. (57). A total of 102 patients were randomized to TURBT with WLI or PDD with 5-ALA. Study groups were similar except that more patients with solitary tumors were randomized to TURBT with WLI. Median follow-up in the WLI and PDD groups was 39 and 42 months, respectively. The median time to the first recurrence was 5 and 12 months. Recurrence rates at 2, 12, 36, and 60 months were 41, 61, 73, and 75% on the WLI group, whereas it was 16, 43, 59, and 59% in the PDD group. Among the 51 patients in each arm, progression occurred in 9 and 4 patients, respectively (57).

Grossman et al. reported long-term data on the use of PDD during TURBT (48). This is an extension of their initial pivotal phase III study (36). Patients were followed up for a median period of 53 and 55.1 months in the WLI and PDD groups, respectively (48). Intravesical therapy rates were comparable between the WLI and PDD groups. In the WLI group, 31.8% of patients remained tumor-free, whereas 38% remained tumor-free in the PDD group. In the WLI group, the median time to recurrence was 9.4 months compared with 16.4 months in the PDD group ($p = 0.04$). Cystectomy rates were lower in the PDD group than in the WLI s at 4.8 and 7.9%, respectively ($p = 0.16$). This may indicate a trend toward improved bladder preservation in the PDD group (48).

Denzinger et al. published their data on recurrence rates with an 8-year follow-up (58). In this study, 5-ALA was used as the photosensitizing agent. A total of 301 patients were recruited; of them, 191 were evaluable for efficacy. The patients were followed up for a median period of 83 and 86 months in the WLI and PDD arms, respectively. In the WLI group, recurrence-free survival was 73, 64, 54, and 45% at 2-, 4-, 6-, and 8-year follow-up; for the PDD group it was 88, 84, 79, and 71%, respectively, favoring TURBT with PDD ($p = 0.0003$). The beneficial effect of PDD was maintained across all prognostic groups irrespective of the use of intravesical therapy. In this study, the authors, however, did not find a significant beneficial effect of PDD TURBT on preventing progression to the muscle-invasive stage (58).

The impact of PDD TURBT on oncologic outcome after radical cystectomy was reported by Gakis et al. in a retrospective study (59). The authors retrospectively evaluated the effect of PDD (ALA or HAL)- guided TURBT on the outcome of 243 consecutive radical cystectomies. In univariate analysis, the PDD TURBT group had a higher median number of TURBTs before radical cystectomy, a higher number of re-resections, more frequent BCG treatment, and longer intervals between first TURBT and radical cystectomy as well as a lower rate of adjuvant chemotherapy. Three-year recurrence-free survival, cancer-specific survival, and overall survival after TURBT were significantly better in the PDD with HAL TURBT group. In addition to the pathologic tumor stage, nodal stage, and surgical margins, the performance of TURBT with HAL guide was an independent predictor of recurrence, cancer-specific death, and overall death after radical cystectomy (59). HAL-guided TURBT was found to be an important predictor of improved survival after radical cystectomy for the first time in this study (59). In contrast, May et al. in their multi-institutional retrospective study, did not find a significant impact of PDD during TURBT on prognosis after radical cystectomy (60).

Yuan et al. reported a meta-analysis of 12 RCTs including 2,258 patients. PDD TURBT had lower recurrence rates, delayed recurrence, and improved recurrence-free survival at 1 and 2 years (61). Nine studies in this meta-analysis reported data on progression to the muscle-invasive stage. No significant difference was found between WLI and PDD in progression rates (61).

The definition of progression in studies has been imprecise and inconsistent. Various definitions indicating progression by different investigators are development of muscle-invasive (T2) disease, development of CIS, uncontrollable CIS, nodal metastases, distant metastasis, increase in tumor stage, increase in tumor grade, and disease-worsening requiring treatment change like the need for cystectomy, radiation therapy, or systemic chemotherapy (62). Because of some of these imprecise definitions of progressions, statistically significant progression endpoints have not been achieved in some bladder cancer trials. This issue was discussed in detail by the International Bladder Cancer Group (IBCG). The authors suggested new definitions of NMIBC progression, namely, "an increase in T stage from CIS or Ta to T1 (lamina propria invasion), development of T2 or greater or lymph node (N+) disease or distant metastasis (M1), or an increase in grade from low to high" (62). In view of these new definitions of progression, Kamat et al. reanalyzed their previously published data (36). In the reanalysis (63), TURBT with PDD showed a trend toward lower progression in PDD compared to WLI. The new definitions identified more patients at risk of progression in the WLI and PDD groups. Time to progression was significantly prolonged in the PDD group *vis a vis* the WLI group. A lower rate of progression was particularly evident in progression from Ta to CIS. This is important, as detection rates for CIS are quite poor in WLI. Improved progression rates in the PDD group were unrelated to intravesical BCG treatment, as the BCG rates in both groups were comparable (63).

Cost-Effectiveness

High recurrence rate, low mortality rate, and need for long-term follow-up make bladder tumors the costliest to treat. PDD is beneficial in cost savings. Although there is an initial cost in acquiring new equipment and recurrent cost of HAL, reduced

recurrence rates, reduced need for intravesical instillations, and less intensive follow-up regimen lead to net savings of GBP 45,500 per 100 new patients with NMIBC (64). Daniltchenko et al. performed a cost analysis of 5-ALA-based TURBT based on several procedures required, hospitalization, cost of ALA, and equipment amortization. The authors reported savings of USD 425 per patient per year with PDD (57). In a similar analysis with a median follow-up of 7 years, Burger et al. reported savings of EUR 168 per patient per year with PDD TURBT (65). Malmström et al. through a decision analytical model, projected that using HAL PDD for all first TURBTs and all recurrence TURBTs in 1 year would lead to net savings of SEK 1321716 to the Swedish health exchequer (66). Garfield et al. (67), in their cost-effectiveness study on HAL PDD using a probabilistic decision tree model, reported reduced costs with the use of PDD compared to WLI alone ($25,921 with PDD vs. $30,581 with WLI) (67). Using PDD throughout the management of NMIBC leads to significant cost savings and improved quality-adjusted life years (68).

PDD: Current Place

Photodynamic diagnosis is the most extensively studied macroscopic image enhancement technique. PDD-directed TURBT leads to more complete TURBT and reduced recurrence rates, and improved recurrence-free survival. It decreases recurrence rates more so in patients with high-risk lesions. However, most data on progression use an imprecise definition of progression. Reanalysis of data with a new precise progression definition suggests beneficial effects of TURBT with PDD on delaying progression. PDD may have a beneficial effect on post-radical cystectomy outcomes.

It is still not used widely because of the expensive equipment and recurring cost of a fluorescence agent. One survey from German-speaking countries reported that WLI was only performed by 60.2% of practitioners, with additional PDD performed by 36.8% (69). In a large Chinese study comprising 14,260 patients, around 74.3% of the patients were diagnosed by WLI alone. PDD had an additional detection rate of 1% (2). Moreover, its low specificity leads to additional biopsies and increased anxieties. Also, it is not yet available in many countries (14).

Narrow-Band Imaging

Principle

Narrow-band imaging is a high-resolution endoscopy technique that filters out the red spectrum of light and selectively transmits blue (415 nm) and green (540 nm) segments of white light without actually utilizing any intravesical contrast medium (1, 3, 10). As the blue and green spectra coincide with absorption peaks of hemoglobin (oxy-hemoglobin: 415, 542, 577 nm and deoxy-hemoglobin: 430 and 555 nm), tissues rich in hemoglobin content will have a different appearance compared to adjacent tissues. This contrast helps clinicians to predict the possibility of neovascularized malignant tissue in a better manner than conventional white light cystoscopy. Mucosal capillaries are accentuated by the blue segment (415 nm), and relatively deeper located submucosal capillaries appear cyan by the green segment (540 nm) of filtered light.

Technical Consideration

Narrow-band imaging is available either as an integrated video cystoscope or a camera head that can be fitted to a telescope. There is no need to instill an exogenous contrast. The NBI mode would highlight the microvasculature to identify neovascularisation and would enable the clinician to find out about the boundary between vascularized and non-vascularised structures.

Narrow-band imaging provides a better view of the urothelium. The vasculature appears dark green to black. The normal mucosa looks pale white (70). The contrast between flat growths and normal mucosa is enhanced, leading to improved diagnosis. It sometimes becomes difficult to distinguish between flat and papillary lesions with NBI (71). As expected, NBI produces suboptimal images in the presence of hematuria and inflammation (72, 73). Moreover, it should always be combined with WLI cystoscopy to avoid false positives (73). NBI is useful not only in judging margins of resection but also in rapidly identifying lesions that could have been missed on WLI. Carcinoma *in situ*, an aggressive variety of urothelial cancer, would certainly be seen in an efficient manner because of a characteristic appearance based on higher vascularity.

Initial primary versions of NBI scopes had relatively lesser illumination than white light, making it difficult to be used as the primary method of diagnosis; however, in 2013, second-generation NBI scopes (EVIS LUCERA ELITE and EVIS EXERA III, Olympus) were manufactured and had high illumination intensity (10). In the third-generation NBI system (EVIS X1), Olympus, Japan designed two LED lights with specific central wavelengths of 460 and 540 nm to provide a narrow bandwidth (10). This modification helped increase the longevity of the light source while reducing the overall volume of the system. NBI technology is now incorporated into flexible scopes as well for pure diagnostic office-based procedures to pick up recurrences and flat lesions in patients under regular surveillance for bladder cancer (1).

Learning Curve

There is no significant learning curve with NBI. Bryan et al. reported on their experiences in NBI being performed by a "new user" (71). A trainee only underwent one session of training and observation of the technique. In this series, the new user detected 0.65 additional lesions with NBI as compared with WLI (71). This is not statistically different than the number of lesions detected by an experienced user (74). In another study on evaluation of 50 cystoscopy images by WLI and NBI shown to independent observers, minimal individual variation was seen among the observers. Detection rates between experienced users and novice were not different (75). This suggests that NBI cystoscopy is easy to learn and master.

Role in Diagnosis

The first clinical experience in performing NBI cystoscopy was published by Bryan et al. (74). In this study, the authors

performed NBI flexible cystoscopy on 29 patients diagnosed as having recurrent bladder cancer that has been previously diagnosed by WLI. The NBI examination detected a mean of 0.52 additional tumors in this small cohort. These additional tumors might have been labeled "early recurrence" on subsequent WLI cystoscopy. However, this study did not study CIS lesions. Herr and Donat reported their diagnostic cohort study on WLI vs. NBI (70). The authors first performed WLI cystoscopy and then NBI cystoscopy on patients with suspected recurrent NMIBC. NBI was able to diagnose more lesions than WLI, more so CIS. WLI detected 2.3 lesions per patient, whereas NBI detected a mean of 3.4 lesions in this cohort (70). Around 13% of the patients underwent unnecessary biopsies because of positive NBI.

Chen et al. improved the patient-level detection rate of NBI compared to that of WLI (97.9 vs. 88.8% $p = 0.002$). Tumor-level detection rates were 96.8 and 79.3%, respectively. False positive rates were not different in the NBI and WLI groups (76). Cauberg et al. reported a study wherein (77) each patient was evaluated by WLI and later by NBI in the same sitting by a different surgeon. NBI detected a mean of 2.1 lesions per patient, whereas WLI detected 1.7 lesions ($p < 0.001$). NBI had a detection rate of 94.7%, and WLI had 79.2%. The false positive rates of NBI were higher than those of WLI. NBI detected additional tumors in 35.9% of the patients. The detected additional tumors in this series were mainly grade 3 lesions.

Ye et al. reported their experiences in a multicentric randomized trial of WLI and NBI (78). The authors reported higher sensitivity with NBI and superior early bladder tumor and CIS detection (78). The specificity and false positive rates in the NBI arm were superior to those of the WLI examination but were not statistically significant (78). Giulianelli et al. in a comparative study, reported improved detection by the NBI arm by 30%, especially in patients with lesions smaller than 3 cm, and unifocal and recurrent lesions (79). Geavlete et al. reported on their experiences in a single institute study on within-patient comparison of WLI and NBI (80). Detection rates of NBI were better than those of WLI for papillary and CIS lesions. False positives were slightly higher in the NBI but not statistically significant. Moreover, NBI was useful in detecting positive tumor margins (80).

Tatsugami et al. (72) et al. also reported superiority of NBI-assisted diagnosis for detection of bladder tumors and CIS lesions, and in patients with positive and negative urine cytology findings. The reduced specificity of NBI in this series may be due to false detection of inflammatory lesions as cancerous by NBI. Zhu et al. reported a case series of twelve patients with positive or suspicious urine cytology (81). Patients in whom outpatient cystoscopy with WLI and imaging did not detect any bladder lesion were only included in the case series. Flexible cystoscopy was performed first with WLI and then with NBI. In this group of patients, sensitivity and specificity were 78 and 91% for NBI vs. 50 and 80% for WLI (81). In a single blind study on TURBT with NBI and with WLI from Korea, Kim et al. (82) reported better diagnostic yield with NBI than with WLI (85.5 vs. 80.9%). One-year recurrence-free rates were 85.2% with NBI and 72.2% with WLI. However, these recurrence-free rates were not significantly different (82).

Jecu et al. in a retrospective analysis, reported significantly improved detection rates of papillary NMIBC and CIS (94.9 vs. 88.1% and 95.7 vs. 65.2%) (83). NBI cystoscopy resulted in better diagnostic accuracy with NMIBC and CIS. Additional tumor detection rates for NBI were 56.6 vs. 8.7%, 28 vs. 10.3%, 30.3 vs. 10.6%, and 31.6 vs. 9.4% in patients with CIS, pTa, pT1, and NMIBC, respectively (83). One of the advantages of performing NBI at the end of an extensive TURBT for a tumor larger than 3 cm is the identification of infiltration of the bladder mucosa at the periphery of resection. Such infiltration is likely to be missed by WLI (84).

Drejer et al. investigated the effect of performing a flexible cystoscope with a high-definition (HD) camera system (85). The authors also evaluated the impact of NBI on the practical management of bladder cancer. Additional NBI findings were not considered clinically relevant. NBI changed the clinical decision in 1.9% of patients when HD cystoscopy was used as standard. However, HD cystoscopy also had high detection of non-malignant lesions (85).

It is common for NBI studies to study the bladder by WLI first and then by NBI (70, 72, 74). It can be argued that the better performance of NBI in these studies is the effect of the "second look" procedure, which adds to the performance of WLI, thereby improving the diagnostic efficacy of NBI. Moreover, most of the prior studies were concerned with the detection of recurrent lesions. This issue was addressed by Shen (86). In this single-center study, two highly experienced blinded observers performed cystoscopies with WLI or with NBI on a random imaging sequence (WLI followed by NBI and vice versa), thereby negating the effect of a second look. The study only enrolled primary suspected lesions that have not been biopsied or resected previously or have not received any intravesical therapy. In this controlled study, the sensitivity of NBI was significantly better than that of WLI, 92.9 and 77.7%, respectively. However, the specificity of NBI was lower than that of WLI, 73.5 and 82.7%, respectively. It is interesting to note that there were few lesions that were only detected by random biopsies and not by NBI or WLI. NBI was quite superior in detecting CIS. There were no CIS lesions that were detected by WLI and missed by NBI. Here, also, few CIS lesions that were detected by random biopsies were missed by both WLI and NBI. There has been a concern that most additional lesions detected by NBI are low-grade lesions. However, this is not the case (87).

Li et al. reported a meta-analysis of 1,040 patients across seven studies (88). This meta-analysis found NBI to be better than WLI in patient-level and tumor-level analyses. NBI detected lesions in an additional 17% of the patients and an additional 24% of the lesions (88). Zheng et al. reported similar improved detection rates on per-person analysis in their meta-analysis comprising 1,022 patients across eight studies (89). Another meta-analysis of twenty-five studies comprising 1,557 patients was published by Xiong et al. (90). NBI improved compared with WLI, with additional detection rates of 9.9 and 18.6% in per-patient and per lesion analyses. The additional detection rates in this meta-analysis were lower than those in the Li et al. meta-analysis (88) mentioned above.

In diagnosing bladder cancer, CIS is of particular importance. It is more often than not missed by WLI and optical

enhancements come in as a handy tool for the detection of CIS. Most studies report data on CIS separately because of its potential risk of progression. Studies by Herr and Donat (70), Shen et al. (86), Tatsugami et al. (72), and Geavlete et al. (91) have all reported significantly improved detection of CIS lesions by NBI. A meta-analysis by Lee also reported improved detection of CIS lesions by NBI. NBI was able to detect an additional 28% CIS lesions. False positive rates between NBI and WLI were not significantly different (88). A meta-analysis by Xiong et al. (90) reported additional detection rates of 25.1% on per-patient and 31.1% on per-lesion analysis by NBI compared to WLI. **Table 2** highlights the sensitivity and specificity of NBI and WLI.

Effect on Recurrence

Herr et al. reported their initial data on long-term follow-up of newly diagnosed low-grade, non-invasive papillary tumors (92). Of the original group of 215 patients, 126 were followed up by regular cystoscopies. These patients were later followed by WLI and NBI when NBI became available (93). Tumor recurrence rates for 3 years before and after NBI became available were compared. The authors reported fewer patients with recurrence, fewer number of recurrent lesions, and longer recurrence-free survival by NBI (93).

Performing TURBT entirely with NBI is feasible (94). Moreover, performing additional NBI-directed biopsies on patients with high-grade lesions at the end of extensive second TURBT leads to identification of missed high-grade lesions (84). A frequency-matched index control study on NBI vs. WLI TURBT was reported by Cauberg et al. (95). In the study group, all lesions detected by NBI or WLI were resected, and the control group was the retrospective matched group. Residual tumor rates at first follow-up cystoscopy were significantly less in NBI than in WLI (15 vs. 30.5% OR: 2.7, one-sided 95% CI: 1.2–6.1; $p = 0.03$) (95). Naselli et al. published their randomized trial of TURBT with WLI or with NBI (96). In this study, a complete procedure was performed with either WLI or NBI. Follow-up cystoscopies were also performed with same imaging modality. Patients were followed up for a period of 1 year. The bladder cancer detection rate of NBI was superior to that of WLI (1.55 vs. 1.36 lesions per person, $p = 0.07$). False positive rates were higher in NBI (28%) than in WLI (21%) but not significant. NBI reduced 1-year recurrence risk by at least 10% (96).

Naito et al. reported results of the Clinical Research Office of the Endourological Society (CROES) randomized trial of TURBT with WLI or with NBI (97). Patients with primary tumors were enrolled and randomized to TURBT with either WLI or NBI. Follow-up cystoscopies were performed with WLI. Surgery with NBI took a longer time. At 1-year follow-up, there was no difference in recurrence rates in the WLI or NBI group. Recurrence rates in low-risk lesions were, however, lower in the NBI group. But that was not the case with the intermediate and high-risk groups. With regard to the intermediate and high-risk groups, it is the biology of the tumors over which NBI does not have any control (97).

T1HG tumors have a poor risk of recurrence. Giulianelli et al. evaluated the use of NBI technology on resection of tumor

TABLE 2 | Characteristics of sensitivity and specificity of NBI and WLI.

Reference	Detail	NBI sensy %	NBI Spey	NBI FPR	NBI PPV	NBI NPV	WLI Sens	WLI Spec	WLI FPR	WLI PPV	WLI NPV
Ye et al. (78)	Pt level	97.7	50.0	50.0	91.4	80.0	66.67	25.0	75.0	82.86	12.12
Ye et al. (78)	Lesion level	98.8	60.90	39.10	76.04	97.59	75.45	58.65	41.35	69.61	65.55
Bryan et al. (74)		100					96.6				
Herr and Donat (70)		100	81.8				87.4	85.8			
Cauberg et al. (77)		94.7	68.4				79.2	75.5			
Tatsugami et al. (72)	BldT	92.7	70.9		63.4	94.7	57.3	86.2		69.2	78.8
Tatsugami et al. (72)	CIS	89.7	74.5		78.8	87.2	50.0	83.6		76.3	61.3
Shen et al. (86)		92.9	73.5				77.7	82.7			

margins and assessed the impact on tumor persistence in re-TURBT (98). Tumor persistence rate was 19%, which was lower than that of WLI. Moreover, none of their patients had pT2 disease in re-TURBT (98). Kobatake et al. reported a comparative study on NBI vs. WLI (99). Patients in respective groups underwent cystoscopy, TURBT, and follow-up cystoscopies with the same modality. No additional treatment was offered to these patients except for multiple biopsies and resection of recurrent lesions. NBI had higher sensitivity than WLI (95 vs. 70%; $P < 0.01$). Also, NPV was better with NBI (97.1 vs. 86.8%; $p < 0.01$). One-year recurrence rate with NBI was 21.1%, whereas with WLI it was 39.7% ($p = 0.016$), suggesting a beneficial effect of NBI on diagnosis as well as recurrence rates (99).

A meta-analysis by Xiong et al. (90) reported significantly reduced recurrence rates with NBI at 3 and 122 months. The outcomes of these trials make us believe that NBI TURBT has some role in delaying tumor recurrence (100). Kang et al. published a meta-analysis of RCTs performing NBI and WLI during TURBT (101). The authors reported significant benefit in recurrence rates at 3 months, 1, and 2 years. The risk ratios were 3-mo RR: 0.39 (95% CI, 0.26e0.60; $p < 0.0001$), 1-yr RR: 0.52 (95% CI, 0.40e0.67; $p < 0.00001$), and 2-yr RR: 0.6 (95% CI, 0.42e0.85; $p = 0.004$) compared with WLI TUR (101).

NBI After BCG Therapy

The reddish patches seen after intravesical BCG can be mistakenly considered as tumors. Switching to NBI, the suspicious neovascularized lesions look brownish and black, whereas lesions that look greenish with NBI are less likely to be tumors. Herr evaluated the utility of NBI in a post-BCG setting (102). In 21 out of 22 patients, NBI correctly identified a tumor. The false positive rate of NBI was 32% in this study. NBI outperformed urine cytology in detecting patients with persistent lesions. This would have a great prognostic implication in this high-risk group of patients in identifying patients who would be candidates for more intensive therapy (102). The results published by Herr (102) were, however, not replicated by other researchers. It has been argued that NBI in a post-BCG setting may lead to more unnecessary biopsies, especially if the duration between last BCG instillation and cystoscopy is shorter (103). In another review, Herr proposed deferring a 3-month biopsy in post-BCG patients with negative cytology and benign-looking lesions (104).

Therapeutic Impact of NBI and Effect on the Progression

It is proposed that better visualization, improved staging, better local control, and fewer recurrences with NBI would translate to an improved therapeutic impact of NBI (104). Despite this, its effect on the meaningful endpoint is unknown (78). Shadpour et al. applied the EORTC scoring system to tumors detected by WLI and NBI in the same cohort of patients. Progression risk scores were not statistically different between NBI and WLI (87). More prospective studies are needed to evaluate the beneficial effect of NBI on the ultimate therapeutic outcome (104).

Cost-Effectiveness of NBI

There are limited data on the cost-effectiveness of NBI. In one review, NBI resulted in estimated savings of 230-500 USD per year; probably a result of the reduced recurrence rates. However, they excluded the cost of pathological analysis and prolonged operation times (105).

Narrow-Band Imaging vs. Photodynamic Diagnosis

In contrast to PDD,which depends on stronger absorption and extended excretion of protoporphyrin by cancer cells rather than normal bladder tissues; NBI is not cancer-specific. It depends on the improvement of the morphological visibility of the superficial epithelium (106). Interpretation of NBI is subjective.

A recently published meta-analysis including 4,519 patients by Motlagh et al. that focused on enhancement techniques during TURBT in NMIBC found that performing PDD during TURBT with concurrent single immediate intravesical chemotherapy (SIIC) resulted in superior recurrence outcomes. A significant reduction in 12- month recurrence rate performing PDD along with an increase in risk benefit by an additional 32% reduction in odds ratio was noticed when using concomitant SIIC (107).

Naya et al. were the first to report a comparative study on PDD with oral 5-ALA and NBI simultaneously in the same patients (108). In this study, 10 patients were included. All underwent WLI cystoscopy first, followed by NBI, and then PDD in an alternating sequence. Patients with abnormal cytology and undefined papillary mucosa were recruited. The results were more in favor of PDD. PDD detected all cancer lesions and missed 4% of dysplasia. NBI missed 5% of CIS and 10% of dysplasia. Sensitivity for Cis and dysplasia detection was 91.6% for PDD and 62.5% for NBI (108). Another larger study reported by Drejer et al. recruited 171 patients (109). Patients underwent cystoscopy with WLI followed by NBI and PDD. In the patient-level analysis, compared to WLI, NBI and PDD showed a significantly higher sensitivity for detection of CIS and dysplasia. The sensitivities were NBI 95.7%, PDD 95.7%, and WLI 65.2% ($p < 0.05$). But the specificities were not statistically different (NBI: 52, PDD 48, and WL 56.8%). On per biopsy analysis, NBI and PDD both had better sensitivity than WLI. Per lesion sensitivities were 72.2, 78.2, and 52.7% for NBI, PDD, and WLI, respectively ($p < 0.05$). PPVs were not different among these modalities (NBI 23.7, PDD 22.2, and WL 19%). NBI was suggested as a valid alternative to PDD by these authors (109).

Kwon et al. published a network meta-analysis of therapeutic outcomes of TURBT guided by WLI, NBI, PDD ALA, and PDD HAL (110). The authors found that the recurrence rate of TURBT with 5 ALA was lower than that of TURBT with HAL guide (OR = 0.48, 95 % CI 0.26–0.95). Theoretically, HAL has better penetration and accumulation in neoplastic cells, but 5-ALA has been evaluated in more studies in this meta-analysis. This might be the reason for the superiority of 5ALA over HAL in this meta-analysis. Recurrence rates with ALA- and NBI-guided TURBT were similar. All three imaging modalities, i.e., ALA, HAL, NBI, had lower recurrence rates than WLI TURBT. But there were no significant differences in progression rates (110). NBI had the highest rank probability for progression-free rate. The authors

also suggested that NBI would be preferable in patients who have undergone intravesical instillations (110).

Chen et al. published a meta-analysis of 26 studies including 3,979 patients. The studies used WLI as the index imaging modality and PDD with HAL or 5-ALA or NBI as the comparator imaging modality (111). In the lesion-level analysis, pooled diagnostic odds ratio (DOR) was highest with HAL at 78.14 (95% CI 31.42–194.28), followed by NBI at 40.09 (95% CI 20.08–80.01). DOR was lowest with 5-ALA at 18.14 (95% CI 4.28–76.87). Higher DOR indicates better performance of the test, and HAL performed better here. Area under the receiver operating curve (AUROC) was, again, best with HAL (0.94) (95% CI 0.92–0.96) followed by NBI (0.88) (95% CI 0.85–0.91), and 5ALA (0.82) (95% CI 0.79–0.85). Pooled sensitivities of HAL, NBI, and 5-ALA were 0.95, 0.94, and 0.9, respectively. Specificities were 0.81, 0.79, and 0.69, respectively. In the patient-level analysis, NBI had the best DOR at 358.71 (95% CI 44.5 to 2,891.71) followed by HAL at 59.95 (95% CI 24.3–147.92), and 5-ALA at 79.52 (95% CI 0.94–6,759.92). The authors proposed that NBI could be the most promising diagnostic modality for NMIBC looking at cost, reliability, and simplicity (111).

Current Place

Narrow-band imaging significantly improves bladder cancer management with improved detection rates and reduced recurrence rates. The lower recurrence rates are irrespective of intravesical instillation (112). It is likely that it would lead to more relaxed surveillance. It is easy to learn and cost-effective. Considering cost, reliability, and simplicity, it may be a promising modality (111). However, it is not as extensively studied as PDD.

Image 1 S

Technical Consideration

In image 1S, images are processed on a software platform to give different contrast specifications. Different light wavelengths are used to produce images with different contrast specifications. A white light image is enhanced by two modalities, Clara and Chroma. Clara enhancement creates a clearer image of darker regions by local brightness adaptation. Chroma enhancement improves the sharpness of an image by enhancing local color contrast. These modes can be used together as Clara + Chroma. In addition to this, there are two predefined post-processing virtual chromoendoscopy enhancements called spectra A (SA) and spectra B (SB). In these SA and SB modes, different color contrast is generated by changing the effective spectral response in an image (113). The original WLI image and the enhanced mode are shown in the endoscopy monitor side by side in real time, in contradistinction with PDD or NBI (114).

In the spectra A (SA) mode, the green and blue light signals from RGB signals are separated. The contrast of capillaries and vessels in the superficial mucosa and submucosa is highlighted in the SA mode. In the spectra B (SB) mode, there is no color transformation, but a color tone shift occurs. In this mode, in addition to the vessels in superficial mucosa and submucosa (which are highlighted in SA), deeper tissue layers are also visible (113). Using SB may be beneficial in case of visual interferences because of hematuria (11).

Kamphuis et al. evaluated the image enhancement capabilities of SA and SB under three different layer thickness values of 100, 200, and 300 um. For superficial layers, both SA and SB showed increased absorbance, whereas for intermediate layers SA and SB both showed more absorbance than white light. Absorbance was significantly higher with the SB mode in the intermediate layer. For deeper layers, both white light and SB were more sensitive to absorbance than SA. The authors hypothesized that SA and SB would help in the differentiation of normal mucosa and tumors (113).

Kamphuis et al. evaluated differences in interpretation of the bladder urothelium imaged with image 1S enhancement modes on iPad app (115). Cystoscopy images were recorded in WLI, SA, and SB, as well as Clara and chroma combined modes. They selected 20 bladder areas for the study, thereby giving 80 images. These images were shown to 73 participants on iPad. The participants were asked to delineate abnormal-looking areas with a stylus. Instead of histological diagnosis, the assessment of images by a panel of urologists at the Academic Medical Center, Amsterdam, the Netherlands was used as the control. The panel classified these images as easy to delineate (agreement) and difficult to delineate (disagreement). Observers graded the quality of images with image 1 S enhancement to be better than WLI. In this study, the authors observed less variation in interpretation in chroma + clara and SB than WLI and SA in easy to delineate cases. The quality of images in enhancement modes was graded significantly better by the participants (115).

Learning Curve

Soria et al. presented a conference study evaluating the effect of a surgeon's experience on the correct cystoscopic diagnosis of bladder cancer by WLI, PDD, and image 1S (116). Twenty-six patients with prior history of high-grade bladder cancers or positive urine cytology and negative ultrasound were included. Cystoscopies were performed by a senior urologist with long-term experience in PDD. Video recordings of these cystoscopy procedures were shown to urology residents not experienced in PDD or image 1S, and they were asked to map areas where they would take a biopsy. The residents' remarks were compared with the procedures performed by experienced urologists. A lower rate of missed bladder cancer lesions by PDD as well as image 1S irrespective of the experience of the surgeons was noted. The authors concluded that both PDD and image 1S would help in decreasing the chance of false negative cystoscopies even with less experienced observers. In this study, the interobserver agreement rate did not differ among WLI, image 1S, and PDD (116).

Role in Diagnosis

Chondros et al. presented the preliminary results of their ongoing study on image 1S in a conference article (117). In this study, each patient underwent cystoscopy with WLI and the SB mode with random allocation by two independent experienced urologists. Enhanced cystoscopy identified significantly ($p = 0.003$) more (48/78, 61.5%) lesions than WLI (37/78, 47.8%). The advantages of image 1 S were better viewing experience and better identification of suspicious lesions. This method does allow for a within-patient comparison of WLI and S enhancement modes.

There was a better correlation of positive urine cytology with SB mode findings but not with WLI. The SB mode enhancement was found to be superior to WLI in the follow-up setting (117).

Soria et al. reported their experience in bladder cancer diagnosis of patients with prior history of high-grade bladder cancers or positive urine cytology and negative ultrasound (116). Compared with WLI, image 1 had a lower rate of missed lesions. The overall concordance rate was also better in image 1S than in WLI. This study, however, neither compared the performance of image 1S or PDD with that of histological diagnosis, nor did the abstract mention which of the image enhancements was used in the recordings (116). Mulawkar et al. published their findings of a web-based survey on cystoscopy images in WLI and SA and SB modes (118). Cystoscopy images from patients with known or suspected bladder cancer and some patients with recurrent urinary infections, invisible hematuria, and storage or voiding symptoms were included. Side-by-side cystoscopy images were cut into two parts, with the first part being WLI and the second being SA or SB. A total of 10,786 observations were included in the analysis. In this study, the side-by-side images were cut into two parts, WLI and SA or SB. The individual images were shown to observers in a random fashion. In patients without cancer, SA or SB enhancement did not add much to the diagnostic accuracy of WLI. But in patients with cancer, SA and SB added significantly to the diagnostic accuracy of WLI. Negative SA and SB ruled out bladder cancer more effectively than WLI (118).

Comparison of Image 1S With Other Macroscopic Enhancement Modalities

Soria et al. presented their study comparing the performance of WLI, PDD, and image 1S (116). Both PDD and image 1S performed better than WLI and were comparable with each other. But this study was not designed to compare PDD with image 1S head-to-head (116).

Effect on Recurrence, Progression

The Clinical Research Office of the Endourological Society (CROES) is currently conducting a multicenter randomized control trial of WLI and image 1 S (119). In this study, the control arm is WLI cystoscopy-assisted TURBT and the study arm is WLI + image 1S-assisted TURBT. Here, randomization is stratified by a multiplicity of tumors, the primary or recurrent status of the tumors, and macroscopic appearance. The authors aim to study recurrence rate and perioperative morbidity. It is postulated that an enhanced image may help the demarcation between a tumor and a healthy tissue, leading to reduced recurrence rates. The results of this study are awaited (119).

Possible Advantages of Image 1S Over NBI and PDD

Lapini et al. published a comparative observational study on PDD vs. WLI (120). In this study, each patient was examined initially by white light and after that by blue light (PDD) cystoscopy. Then, biopsies of the lesions were performed. All cystoscopies were performed by one endourologist. This is a within-patient comparison of both diagnostic modalities. As the same examiner does WLI as well as PDD cystoscopy, this prolongs operative time, and such an evaluation is subject to "second look bias." Geavlete et al. reported a prospective randomized study on PDD and WLI (38). The authors intended to evaluate the impact of PDD on diagnostic accuracy and treatment changes in NMIBC. Herein, one arm underwent standard WLI cystoscopy and transurethral resection of bladder tumor (TURBT). The study arm underwent standard WLI cystoscopy as well as PDD cystoscopy. The lesions detected on WLI underwent standard WLI cystoscopic resection. The lesions detected on PDD only underwent PDD-guided resection. This was followed by a PDD-guided assessment of resection margins. In this trial, the control and study group patients are different.

Stenzl et al. reported a multicenter prospective randomized double blind trial of PDD (49). The study group underwent standard PDD cystoscopy and TURBT. The control group underwent intravesical instillation of sodium chloride instead of 5-ALA. The patients in both groups were comparable but not the same. If a urologist is aware that a better image enhancement modality like PDD is available for bladder inspection after WLI cystoscopy, a bladder examination by WLI might be performed just superficially (42).

The image 1S system would have advantages over the conventional PDD system. In image 1S, there is no need to instill any medication in the bladder prior to cystoscopy with HAL or ALA, a solution must be freshly prepared. This does increase the time a patient remains in a hospital and would increase costs. Intravesical instillation of 5-ALA or HAL is quite safe, but there has been a report of anaphylactic shock attributed to intravesical instillation of hexvix (HAL) (21). The shock manifested 5 h after instillation. It has been proposed to be a non-immunoglobulin E-mediated allergic reaction. In this case, the dwell time of HAL was 3 h. No bladder tumor was detected, and the patient underwent resection of the prostate. Serum tryptase was raised, and skin test for HAL allergy was positive; hence, there is enough reason to believe that anaphylaxis to HAL is possible.

Photodynamic diagnosis also needs procurement of a new camera, telescope, and light carrier. This might be a good proposition for a busy unit but would not be worth the investment if additional equipment in image 1S and PDD is equivalent. There is some time lag in switching from WLI to PDD. In image 1S, both images are viewed on the same screen side by side. This is helpful for the comparison of different features. The quality of video images and motion artifacts (121) with PDD are also worth considering. However, as of today, we do not have robust studies comparing PDD to image 1S.

Herr and Donat published their study on reduced bladder tumor recurrence rates with NBI in patients on surveillance cystoscopy (93). WLI and NBI cystoscopy were done by the same observer. In the initial part of the study, WLI was performed, and later when NBI became available, both WLI and NBI cystoscopy were performed. Here, the patient acted as his own control. The main objection to this study is the potential "second look bias." Naselli et al. conducted a randomized controlled trial of TURBT with WLI and NBI (96). In one group, TUR was performed with WLI and in the other with NBI entirely. Switch from WLI to NBI or vice versa was not allowed. A second TUR, if required, was also performed in the same modality. This study addresses the utility of image enhancement in reducing recurrence rates. But

this study does not tell us about the "within patient" performance of NBI (96).

Cauberg et al. reported on a prospective trial of NBI vs. WLI (77). In this trial, WLI and NBI cystoscopy of the same patient was performed by two independent observers separately in the same sitting. The NBI observer was blinded to the WLI findings. This technique allowed for a "within-patient" comparison of WLI and NBI. It also eliminated the second look bias but at the cost of prolonging the operative time of cystoscopy and anesthesia for the cystoscopy.

There are some theoretical advantages in using image 1S rather than NBI. NBI requires a special light source, whereas image 1S does not need any such investment. While examining the bladder by image 1S, there is no extra time taken to change the light source from WLI to NBI mode. With image 1S, as both the WLI and spectra images are viewed on the same screen side by side, one gets a real-time perception of the extent of a tumor compared to NBI where the old image is not available for comparison after switching to NBI from WLI. This would theoretically help in the proper completion of TURBT. Operative time is saved, and no extra anesthesia time is needed, thereby reducing the risk of the procedure, even if minimal. However, to conclude whether NBI or image 1S is better, more data are needed. If spectra modes in image 1S are reliable and reproducible in the diagnosis of bladder lesions, these modes do have the potential of replacing NBI and PDD.

Current Place

Image 1S enhancement modalities need comprehensive evaluation under actual clinical conditions. Its beneficial role in the diagnosis of bladder cancer is promising. Its exact accuracy, sensitivity, and specificity in a clinical scenario need to be studied. The role of image 1S in the recurrence and progression of bladder cancer is not yet clear. Its exact place *vis a vis* other established modalities like PDD and NBI needs further trials. If not inferior or equivalent to PDD and NBI, it has the potential of replacing these two modalities for the reasons explained above.

Other Emerging Macroscopic Imaging Modalities

Emerging macroscopic techniques are twin monitor-mode PDD, autofluorescence, and scanning fiber endoscope.

A possible shortcoming of PDD is the ability to view only one image at a time and time taken for switching to blue light and generation of an image, thereby losing the reference WLI image. In a comparative study, Fukuhara et al. tried to overcome this issue by side-by-side visualization of reference WLI image and PDD image in real time (122). They used a flexible bronchoscope (off label use) for this purpose. In this system, white light is emitted in the first 1/60 s followed by blue light in the next 1/60 s. These two images are held in a memory chip, and each image is displayed for 1/30 s on a monitor at a frame rate of 30 per s. This system was compared with the conventional PDD system in terms of better sensitivity, specificity, and reduce false positive rates. Further studies on this novel technique are awaited (122).

Schäfauer et al. developed the first prototype for bladder cancer detection based on the principle of ultraviolet autofluorescence (123). This uses endogenous fluorophores in tissues, which are excited by ultraviolet light, and autofluorescence is measured and color-coded. The prototype was able to differentiate between normal urothelium and bladder cancer (123). Kriegmair et al. reported their pilot study on wide-field autofluorescence-guided TURBT (124). In this pilot study, the authors used a D-Light system and customized a band pass filter at the eyepiece of the endoscope. No intravesical instillation is required. Normal urothelium looks greenish, and papillary tumors look brown-reddish. This technique has the potential to increase detection rates of bladder cancer (124).

Scanning fiber endoscope uses an ultrathin endoscope. This can be used as a standalone endoscope or as a probe (125). It can be steered remotely, and images generated from it can be stitched to generate a 3D mode of the urinary bladder (126).

CONCLUSIONS

All these macroscopic image enhancement modalities have proven their utility in improved detection and short-term cancer control. Some studies have shown beneficial effects in terms of delayed progression and improved post cystectomy outcomes with PDD. NBI may be an acceptable alternative to PDD if some of the meta-analyses are to be believed. Most of the image enhancement modalities have not proven their utility in delaying progression or long-term cancer control. Most of these modalities claim to result in more complete TURBT. The presence of detrusor muscle is the surrogate marker for the completeness of TURBT. Data on the effect of an image enhancement modality on the presence of detrusor muscle in TURBT specimens are lacking. Cancer progression and long-term control are governed by the biological nature of cancer cells. PDD may have some role in early detection in this regard. However, well-designed studies are needed to establish the efficacy of these modalities in the evaluation of patients with bladder cancer. The last word in this regard is yet to be written.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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