Comprehensive Diagnostic Approach to Bladder Cancer

Molecular Imaging and Biomarkers

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Foreword

Over the past several years, tremendous advances have occurred in the management of patients with bladder cancer based on an improved understanding of the underlying biology of the disease. The Cancer Genome Atlas Research Network's comprehensive molecular characterization of bladder cancer has provided important insights into the genetic and epigenetic alterations present in urothelial carcinomas. During this same time, major developments in the treatment of patients with bladder cancer have occurred with the incorporation of immune checkpoint inhibitors, antibody drug conjugates, and targeted therapeutics across clinical disease states—including noninvasive, muscle-invasive, and metastatic disease—leading to significant improvements in patient outcomes. Although these advances have been transformative, many more questions than answers remain. In spite of a new understanding of recurrent mutations, copy number alterations, molecular subtypes, the immune microenvironment, and other facets of bladder cancer biology, there is a desperate need to develop and validate biomarkers to select patients for treatment and to better understand their prognosis. Furthermore, next generation imaging has led to paradigm shifts in the diagnosis and management of many malignancies; however, in bladder cancer, conventional imaging studies are still routinely used for diagnosis and management. Ongoing work in bladder cancer is exploring new imaging techniques such as molecular imaging for advanced disease and optical techniques and enhanced cystoscopy for localized disease.

It is clear that in order to advance the field of bladder cancer, the focus must be on the development of tissue and liquid-based biomarkers and new imaging modalities to ensure that we deliver the right treatment to the right patient at the right time. Although the research reviewed in *Comprehensive Diagnostic Approach to Bladder Cancer: Molecular Imaging and Biomarkers* is a testament to the remarkable progress in the field, the momentum in biomarker and imaging-based research

must continue with a goal toward the integration of novel biomarkers and imaging modalities into the screening, diagnosis, and treatment of patients with bladder cancer.

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Chapter 1
Epidemiology of Bladder Cancer: Trends and Disparities

Carissa Chu and Sima Porten

Incidence and Natural History

Bladder cancer is most common cancer globally, with over 500,000 new cases per year [1]. In the United States, it is the sixth most common cancer overall and the fourth most common cancer in men in 2020 following prostate, lung, and colon cancers [2]. Bladder cancer accounts for 4.5% of all new cancer diagnoses, with 81,400 new cases estimated for 2020 in the United States [2]. Bladder cancer is three to four times more common in men than in women, and the median age at diagnosis is 65–70 years [3].

Using statistical models via SEER, age-adjusted rates for new bladder cancer diagnoses have been falling on average 1.2% each year over 2008–2017 [2]. Age-adjusted death rates have been falling modestly on average 0.6% each year over 2009–2018 [2]. Five-year relative survival trends since 2000 are shown in Fig. 1.1.

Bladder cancer incidence and mortality are variable worldwide, as shown on the heat maps in Figs. 1.2 and 1.3. While North America and Europe have the highest age-standardized incidence rates, the highest mortality rates appear to be concentrated in Northern Africa and parts of Europe. Regional differences in exposure to known risk factors such as cigarette smoking, occupational exposures, contaminated drinking water, and endemic chronic urinary infections by *Schistosoma haematobium* are all thought to be responsible for the observed variability in incidence, although access to care and the existence of robust registries are also key [4]. In

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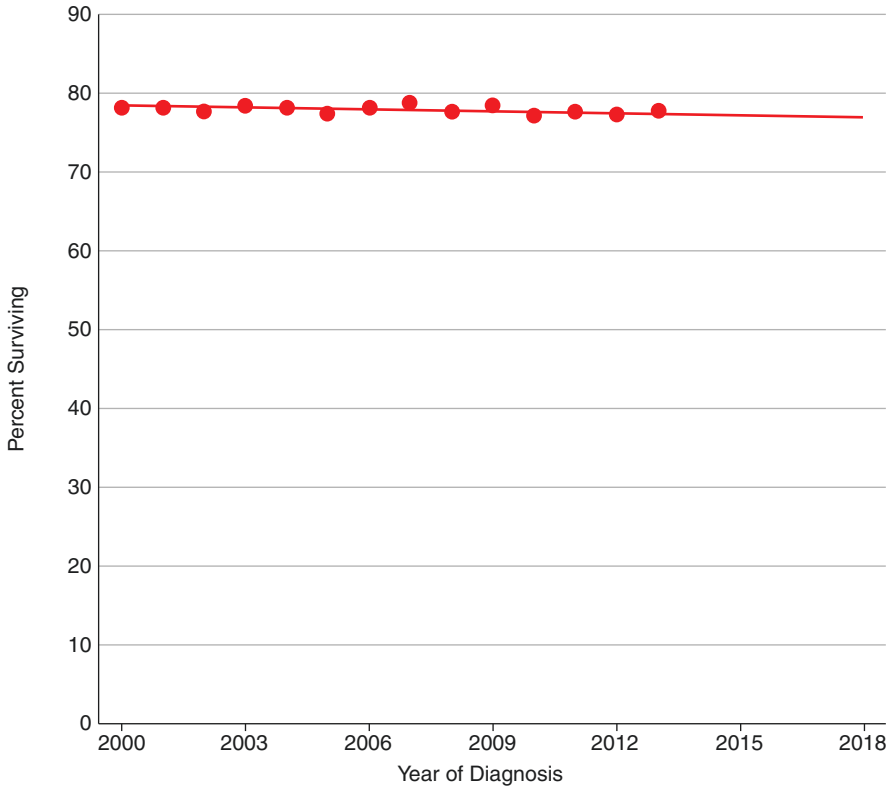

Fig. 1.1 Five-year survival trend of bladder cancer (all stages, genders, ethnicities). (Source: SEER 18 cancer registries. Created by https://seer.cancer.gov/explorer)

contrast, variability in mortality rates is less drastic (Fig. 1.3). Reasons for this are less clear, perhaps related to less ambiguity in reporting deaths secondary to advanced bladder cancer related to muscle-invasive disease [5]. Currently, global efforts to reduce the burden of bladder cancer are centered around smoking cessation [6].

Non-muscle Invasive Bladder Cancer

Malignant urothelial tumors that have not invaded the detrusor muscle layer are termed non-muscle invasive bladder cancer (NMIBC). About 70–80% of bladder tumors are NMIBC at presentation. Of these tumors, 70% are present as stage Ta, 20% as T1, and 10% as carcinoma in situ (CIS) [3].

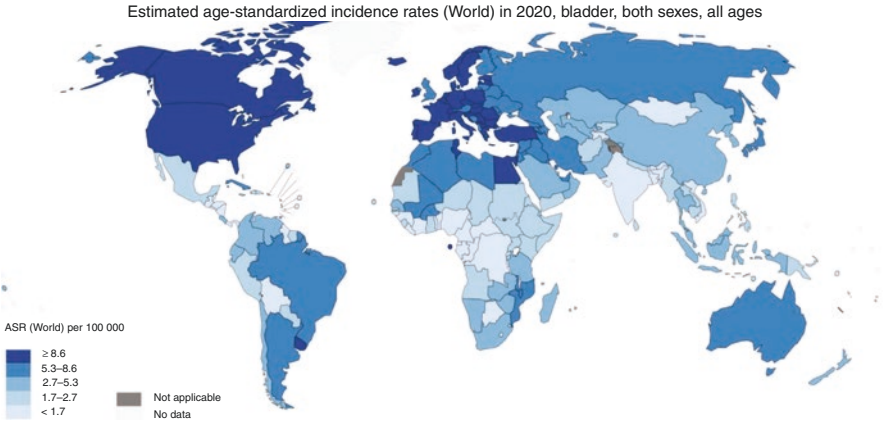

Fig. 1.2 Age-standardized incidence rates of bladder cancer per 100,000 population globally. (Data source: GLOBOCAN 2020 Graph production: IARC (http://gco.iarc.fr/today) World Health Organization)

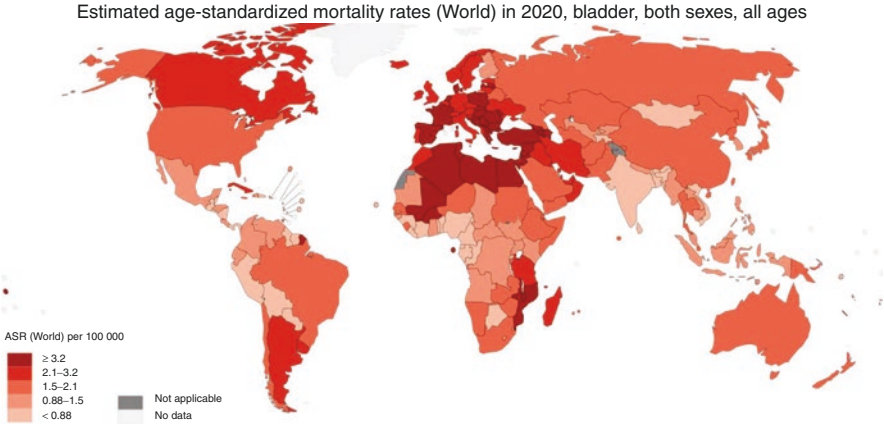

Fig. 1.3 Age-standardized (world) mortality rates (per 100,000/year) of bladder cancer. (Data source: GLOBOCAN 2020 Graph production: IARC (http://gco.iarc.fr/today) World Health Organization)

NMIBC is associated with a high rate of recurrence, with increasing tumor grade predicting progression to muscle-invasive disease. For example, low-grade Ta tumors are associated with a high rate of tumor recurrence (15–70% at 1 year) but a low rate of progression to muscle-invasive disease (<5%). High-grade Ta tumors have a 13–40% chance of progressing to lamina propria invasion and a 6–25% chance of becoming muscle-invasive. T1 tumors have the worst malignant potential in terms of recurrence (80%) and progression (50% within 3 years). CIS is a noninvasive, high-grade tumor by definition and often coexists with other bladder tumors.

CIS is associated with high rates of recurrence (82%) and progression (42–83%) especially if not treated with intravesical therapy.

In the absence of progression to muscle invasion, the long-term disease burden of NMIBC remains high. NMIBC is associated with a high symptom and health-related quality-of-life impact despite intravesical treatment. Disease management incurs high costs to healthcare systems, ranging from $2830 to $9554 per-patient Medicare expenditures per year [7].

Muscle-Invasive Bladder Cancer

Muscle-invasive bladder cancer (MIBC) is an aggressive disease associated with significant morbidity and mortality, ranging from T2 (invasion into muscularis propria) to T4 (local invasion of adjacent organs or musculature). Approximately 20–30% of bladder cancers are muscle-invasive at presentation. Unlike NMIBC, these cancers are biologically aggressive. 5-year overall survival, left untreated, approaches 5% [8]. In a population-based series of patients with newly diagnosed bladder cancer in Sweden, the untreated 5-year incidence of cancer-specific mortality was 86%, compared to 48% for treated patients. Untreated patients also had a higher risk of progression to metastatic disease (hazard ratio [HR] 2.40, 95% CI 1.28, 4.51), all-cause mortality (HR 2.63, 95% CI 1.65, 4.19), and cancer-specific mortality (HR 2.02, 95% CI 1.24, 3.30) [8]. Despite the introduction of neoadjuvant chemotherapy and innovations in extirpative and bladder preservation therapies, however, overall improvements in mortality for localized and regionalized disease have not been achieved in the past decade [9].

Metastatic Bladder Cancer

Approximately 4–5% of patients present with de novo metastatic disease and 50% progress after local therapy for muscle-invasive bladder cancer at 5 years [10]. An estimated 12,500 deaths per year in the United States are attributable to metastatic bladder cancer [11]. Via lymphatic and hematogenous channels, the spread of bladder cancer typically begins in the pelvic lymph nodes followed by the lungs, bones, liver, and brain. Prognosis is poor with cures rarely achieved, and the median survival of patients at diagnosis of metastatic urothelial cancer is 12 months though recently, the introduction of immune checkpoint inhibitors has demonstrated longer median survival compared to traditional chemotherapies.

Variant Histology

Variant histology comprises a heterogeneous group of tumors which include squamous, sarcomatoid, small cell/neuroendocrine, signet ring, micropapillary, and adenocarcinoma. Overall, variant histology is associated with worse overall survival, but these patients are often excluded from clinical trials. Furthermore, the individual characteristics and biology of each variant are not well understood. Interestingly, a study of 314,177 patients in NCDB found that younger patients (less than 40 years old) were more likely to have variant histology and that half of these cases were overrepresented by women who had worse overall survival [12].

National and Global Trends

In the United States, the overall incidence of bladder cancer is slowly increasing, likely due to improved diagnostic accuracy. Between 1973 and 2009, incidence increased from 21.0 to 25.5/100,000 person-years, driven largely by increase in localized and distant disease and paralleled by an equal decrease in unstaged disease [9]. Similarly, 5-year cancer-specific survival rates improved from 73.9% to 81.4% [9]. Population-based data show that while mortality rates for men with localized and regional disease have decreased over time, they have remained stable for women. Other contemporary studies have shown little change in overall or stage-specific relative survival, with underuse of neoadjuvant chemotherapy.

Disparities in the Diagnosis and Treatment of Bladder Cancer

Disparities exist in the diagnosis, treatment, and prognosis of bladder cancer nationally and worldwide. Screening rates can vary widely among patients which can lead to large differences in outcome, even among those with similar disease features. Currently, hematuria (gross or microscopic) remains the only indication for a bladder cancer workup, which consists of cystoscopy and upper tract imaging in the presence of risk factors [13]. A large proportion of patients are delayed in their referral for cystoscopy, and improvements in detection of earlier stages of disease have not occurred in the last three decades. In a recent study, only 42% of patients with documented hematuria with high-risk features were referred for further evaluation [14]. Access to care, lifestyle characteristics, such as smoking and obesity status, as well as education, referral patterns, and insurance status likely contribute to the observed disparities in diagnosis, treatment, and outcome. While differences in tumor biology may exist, nonbiological factors inevitably account for much of the variation, leading to health disparities linked to social, economic, and environmental disadvantage.

Gender

Although bladder cancer is more common among men, women with urothelial cancer are often diagnosed at higher tumor stages and tend to have a worse prognosis [15, 16]. These differences are even larger in squamous cell carcinoma, adenocarcinoma, and sarcoma of the bladder [17]. The stage migration may be due in part to delays in diagnosis, as women are more likely to undergo workup for cystitis and other benign causes before undergoing urology referral for cystoscopy. Women with bladder cancer are twice as likely to be diagnosed with a urinary tract infection than men prior to diagnostic workup [18]. Additionally, differences in referral patterns exist between men and women. At a Midwest managed care organization, 28% of women with hematuria were referred for urologic evaluation compared to 47% of men [19]. According to Medicare data, women experience greater geographic variation in cystoscopy rates than men when restricting to ICD-9 codes for hematuria only [20]. More recent investigations based on linked Surveillance, Epidemiology, and End Results (SEER)–Medicare data have corroborated that women are less promptly referred to a urologist and more likely to experience delays in hematuria evaluation [14].

Regarding overall prognosis, gender-specific differences are also known to exist in favor of men. White men of higher socioeconomic status have significantly longer survival times, compared to their non-White, female counterparts [21]. Even when controlling for stage, however, women appear to fare worse, with a higher risk of cancer-specific death within the first 3 years of follow-up [22]. In a recent study of 6809 patients with nonmetastatic MIBC, women were significantly more likely to receive a cystectomy compared to other bladder-preservation treatments yet found to have worse bladder cancer-specific survival than men, with no differences in overall survival [23].

Differences in hormone exposure, sex steroid receptor expression, social behaviors, environmental factors, and clinical management approach are thought to account for some of these differences. Importantly, hormonal pathways may drive subtype difference and thereby prognosis between men and women—in one study of 1000 bladder tumors, female tumors expressed higher levels of basal and immune-associated genes, while male tumors expressed higher levels of luminal markers and demonstrated higher androgen response activity [24]. A meta-analysis of 2049 patients from 13 retrospective studies found that the androgen receptor (AR) was downregulated in female tumors compared to male tumors, and in high-grade tumors compared to low-grade tumors [25]. Similarly, the estrogen receptor beta (ER) expression was higher in high-grade tumors compared to low grade and in muscle-invasive tumors compared to muscle-invasive. In NMIBC, ER was associated with worse recurrence-free survival [25].

In contrast, it appears that the use of neoadjuvant chemotherapy may equalize some of these differences. In a study of 1031 patients including 227 (22%) women, the female gender was associated with a higher rate of extravesical disease extension at diagnosis, but after administration of NAC, ypT stage was equally distributed between sexes. There were no independent associations between gender with

regard to ypT0N0 or downstaging rates, overall survival, nor cancer-specific survival [26].

Race

In a large cancer registry-based study, black patients presented more frequently with advanced stage and high grade and experienced significantly worse outcomes. Multivariable analysis showed that black race, socioeconomic status, and health insurance status were all independently predictive of poorer survival when controlling for age, grade, stage, and gender [27]. Similar to female patients, black patients have lower cystectomy rates for muscle-invasive disease [28].

Black patients have the poorest cancer-specific survival when compared to whites and other minorities [9]. Even after accounting for age, tumor characteristics, gender, insurance, geography, and dates of diagnosis, black men and women with bladder cancer remain at significantly increased risk for death compared to whites, with black women faring consistently the worst [29]. In a study of over 22,000 patients in the Nationwide Inpatient Sample database, white patients who underwent cystectomy had a mortality rate of 2.8% compared with 4.2% for black patients and 3.9% for Hispanic patients. Black patients were also more likely to have prolonged hospitalization and in-hospital mortality [30]. Treatment differences may impact late-stage mortality more than early-stage mortality, which could explain the persistently higher mortality rates in black patients. With respect to non-muscle-invasive disease, black race, residence in an urban area, and a census area with low median income correlated with a lower-intensity surveillance regimen than recommended by guidelines [31].

Insurance

Compared with those with private insurance, uninsured and Medicaid-insured patients are at least twice as likely to present with regional disease and 60% more likely to have locally advanced disease at diagnosis [32] while less likely to undergo radical cystectomy [28]. Large geographic variations also exist, with lower cystectomy rates in the south and northeast of the United States [28]. Bladder cancer patients who are either uninsured or Medicaid-insured exhibit 50% and 70% increased risks of death compared with privately insured patients, respectively [29]. In a large study of data from the National Cancer Data Base (NCDB) of nearly 29,000 patients, not only black patients were less likely to receive curative treatments than white patients (OR: 0.74; $p < 0.001$), but also patients without insurance, Medicaid beneficiaries, and young Medicare patients when compared to those with private insurance [33].

Geography

Physical access to a high-volume center plays a role in bladder cancer outcomes. In a study of nearly 4000 patients who had undergone radical cystectomy in the United States, distance to treatment facility was associated with delays in time to cystectomy (>3 months), but not cancer-specific or all-cause mortality after multivariable adjustment [34]. In contrast, other studies have shown that delays to cystectomy are associated with worse outcomes [35]. An NCDB study of over 18,000 patients who underwent surgical treatment for MIBC found lower use of neoadjuvant chemotherapy in settings of lower hospital cystectomy volume, treatment at a nonacademic facility, lower patient income, and receipt of partial cystectomy—interestingly, neither gender nor race was associated with the use of NAC [34].

Financial Toxicity

Financial toxicity, defined as the patient-level impact of the costs of care delivered, merits discussion in the management of both NMIBC and MIBC. Bladder cancer is the costliest cancer among the elderly, estimated at nearly $4 billion per year, and has the highest cost of any cancer when categorized on a per-patient basis [36]. NMIBC is particularly costly to treat in the United States, due to the frequency of surveillance, cost of intravesical chemotherapy, and need for repeat endoscopies and subsequent surgical treatment [37].

In a regional survey of 138 patients with bladder cancer, about a quarter of patients endorsed financial toxicity. Patients who were younger, were black, had NMIBC, and had less than a college degree were more likely to report financial toxicity including the inability to take time off work or afford general expenses, resulting in delaying care. Financial toxicity had deleterious effects on perceived physical and mental health, including cancer-specific health-related quality of life and functional well-being [38]. Similarly, a national survey of 226 patients demonstrated that people who were younger, with a household annual income less than $50,000, not retired, or with insurance that was neither Medicare nor employer-paid were significantly more likely to have worse financial toxicity. The majority of these patients would have wished to discuss cost in the context of treatment preferences [39].

Disparities in Survivorship

In recent years, there has been a growing awareness of not only the oncology but also the quality of life ramifications of a bladder cancer survivorship which can be prolonged. Significant declines in health-related quality-of-life (FR-QoL) scores related to physical health, vitality, and social functioning all decline after bladder

cancer diagnosis. There is an unmet need for research, long-term support, and survivorship resources to address this gap. Within survivorship, continued disparities exist based on gender, race, and other factors [40].

Urinary Diversion

Long-term morbidity associated with radical cystectomy is often tied to urinary diversion, which remains the most studied HR-QoL domain for BC patients. Although studies are mixed, continent diversions (CD) such as ileal neobladders are generally linked to better HR-QoL outcomes, and this is largely affected by continence rates [41]. In a national study comparing CD with ileal conduit (IC) use in nearly 70,000 radical cystectomy cases for bladder cancer, white men were more likely to undergo CD compared to female and black counterparts. CD use was also regional, with the highest rates on the West coast, at teaching centers, and large hospitals [42]. Similarly, Hispanic patients were less likely to undergo CD at another high-volume center [43]. Possible causes for these disparities include clinician bias, patient preference, communication barriers, and proximity to high-volume centers, although these factors are not well studied.

Sexual Function

Sexual dysfunction is very common after radical cystectomy. Men after cystectomy have self-reported rates of erectile dysfunction as high as 80–90% with non-nerve sparing techniques and as low as 10–30% after nerve sparing techniques [44]. While much of the anatomical and surgical details of nerve sparing cystectomy in men is derived from decades of study in the radical prostatectomy literature, female sexual anatomy and function are much less defined and lack a standardized approach. Post-cystectomy sexual dysfunction in women can be grouped into female sexual pain disorders and disorders of orgasm due to damage to the clitoral branches of the internal pudendal artery [45]. Decline in sexual function also occurs in patients with NMIBC—in a study of over 200 patients, erectile dysfunction (60%), vaginal dryness (63%), and fear of contaminating sexual partner with intravesical therapy agents (23%) were most commonly reported, with over half of those interviewed endorsing sexual dysfunction [46].

Conclusion

Bladder cancer is associated with significant morbidity and only modest improvements in mortality in the past two decades despite the introduction of neoadjuvant chemotherapy and innovations in definitive local therapies. Progress in improving

cancer survival has been hindered by existing disparities with respect to gender, race, and access to care, resulting in delays to diagnosis and leading to other adverse outcomes. The high financial cost of bladder cancer management and survivorship in the United States cannot be underestimated—a burden that is often shared with the patient. In the upcoming decade, with the emergence of novel therapeutics for bladder cancer, addressing disparities and health-related quality-of-life outcomes among bladder cancer patients will be of utmost importance.

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Chapter 2
Environmental and Genetic Factors Contributing to Bladder Carcinogenesis

Shawn Dason and Nathan C. Wong

Introduction

While performing its physiologic role of storing urine between voids, the urinary bladder has prolonged contact with innumerable substances encountered in the environment and excreted in the urine. The host response to these substances is influenced by additional factors, such as genetic predisposition or underlying medical conditions. Advances in molecular biology and epidemiology have allowed for the characterization of a number of environmental exposures or host factors that promote the development of bladder cancer (Table 2.1). The origin of a single case of bladder cancer is likely a complex interplay between the factors described below, additional etiologies awaiting discovery, and stochastic effect.

Environmental Risk Factors

Arsenic

Excessive exposure to arsenic is associated with bladder and upper tract urothelial carcinomas. Arsenic is classified as a group 1 carcinogen (sufficient evidence of human carcinogenicity) by the International Agency for Research on Cancer [1]. Contemporary exposure to excessive arsenic is most widespread in the drinking

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Table 2.1 Environmental and genetic factors contributing to bladder carcinogenesis

Factor	Source of exposure	Hypothesized mechanism of carcinogenesis
Environmental		
Arsenic	Primarily drinking water	Urinary excretion of metabolites resulting in mutagenesis
Aristolochia	Traditional Chinese medications, bread in endemic areas	Urinary excretion of metabolites resulting in mutagenesis
Diet	Dietary composition	Low fruit and vegetable intake, processed meat intake, fluid intake and type, and low intake of certain vitamins. Molecular mechanisms remain uncharacterized.
Cyclophosphamide	Cytotoxic chemotherapy used for cancer and autoimmune conditions	May involve acrolein, other metabolites, host factors, oxidative stress, and chronic inflammation
Pioglitazone	Glycemic control in diabetes	Uncharacterized
Phenacetin	Analgesic medication	Possibly p-aminophenol mediated
Radiation	Treatment of other cancers or environmental	Free radical formation and subsequent DNA damage
Immunosuppression	Immunodeficiency conditions, immunosuppressant medications	Oncogenic viral infections, impaired ability to eliminate pathogens and resultant inflammation, loss of immunosurveillance
Infection	Environmental exposure	Various mechanisms, see Table 2.2
Smoking	Traditional tobacco products, electronic cigarettes, secondhand smoke exposure	Various carcinogenic compounds and reactive oxygen species
Occupational risk	Many—rubber and dye industries, textile workers, newspaper, coil, oil, gas, metals, aluminum, glass, electrical, truck drivers, miners, marine workers, hairdressers	Polyaromatic hydrocarbon exposure among others—see text for further details
Genetic		
Genetic polymorphisms	Genetic	Polymorphisms associated with detoxification, nucleotide excision and repair, homologous recombination, cell-cycle regulation, and other mechanisms
Germline mutations	Genetic	Double-strand DNA break repair, homologous recombination regulation, mismatch repair

water, although dietary, inhalational, industrial, pesticidal, and pharmaceutical exposures can be significant in certain instances [1].

Arsenic can accumulate in the drinking water from natural or anthropogenic sources. Studies linking arsenic to bladder cancer primarily come from locations where very high (100 ug/l) arsenic concentrations in the drinking water have been

identified, including Taiwan, Chile, Argentina, Bangladesh, and India [1]. In the United States, 350,000 people drink water with arsenic concentrations above 50 ug/l, and 2.5 million people drink water containing more than 25 ug/l [2]. Exposure to high concentrations of arsenic in the drinking water is possible in American populations that drink from private well water as this generally falls outside the regulation of federal or state authorities [3]. It is likely that arsenic is primarily a bladder cancer risk factor when populations are exposed to it at a high level, above the 100–300 ug/l range [4]. It has been difficult to conclusively link lower concentrations with bladder cancer with ecologic studies having mixed results [4, 5].

The pathogenesis of arsenic-induced bladder cancer is likely multifactorial and interacts with other carcinogens like cigarette smoke [5]. Arsenic is a naturally occurring chemical element. Arsenic exposure can be comprised of organic arsenic compounds, inorganic arsenic compounds, or arsine gas, which all have distinct biologic and toxicologic properties [1]. Once arsenic compounds are absorbed into the bloodstream following ingestion, they are methylated by the liver and then primarily eliminated in the urine. Urinary excretion of arsenic is generally about 60–70% dimethylarsinic acid, 10–20% methylarsonic acid, and 10–30% inorganic arsenic compounds [1]. The excretion of arsenic metabolites in the urine likely leads to mutagenesis by oxidative DNA damage and DNA repair inhibition, changes in DNA methylation patterns, aneuploidy, and gene amplification [6]. Animal models have generally demonstrated a clear link between arsenic compounds and bladder carcinogenesis in a dose-dependent manner. The mechanisms of oncogenesis likely differ based on arsenic metabolite. For example, in the rat model, dimethylarsinic acid has a local effect on urothelial cells, resulting in cytotoxicity, necrosis, and abnormal regenerative cell proliferation rather than oxidative DNA damage [7].

Recognition of the carcinogenicity of arsenic emphasizes the importance of a societal infrastructure to test arsenic concentrations and certify wells and other water sources for safe consumption. Fortunately, there are measures that can be taken to reduce arsenic concentrations in the drinking water [8]. These measures may be difficult to export to rural settings drinking from well-water or resource-limited settings, where excessive arsenic exposure risks are likely highest.

Aristolochia

Aristolochia is a genus of herbs used in traditional Chinese medications that causes an interstitial fibrosis nephropathy and is also highly carcinogenic to the urothelium [9, 10]. Interestingly, carcinogenesis associated with aristolochia is more specific to the upper urinary tract than the lower urinary tract [11]. Because the composition of herbal medications is variable and unregulated, demonstrating aristolochia exposure can sometimes be difficult. Nonetheless, because aristolochia is a common component of Chinese herbal medications, this may be an important carcinogen in populations with high use of these medications. For example, a study estimated that

a third of the Taiwanese population had exposure to medications that may contain aristolochia [12].

There is also strong molecular evidence that Balkan endemic nephropathy, a chronic tubulointerstitial nephropathy that predisposes to urothelial carcinoma, is linked to aristolochia exposure [13]. It is thought that the aristolochia present in the wheat fields of villages near certain tributaries of the Danube river contaminates the flour used to prepare homemade bread. Aristolochia is thus inadvertently ingested, leading to Balkan endemic nephropathy [10].

Aristolochia exposure results in detectable aristolactam-DNA adducts in the renal cortex, which can be a reliable biomarker of prior aristolochia exposure. These adducts are found in the majority of Balkan nephropathy and Taiwanese patients with upper tract urothelial carcinoma [10]. Aristolochia is then associated with several specific molecular signatures that appear to be unique to this method of carcinogenesis, including (i) A:T→T:A transversion, (ii) localization of mutated adenine bases on the nontranscribed strand, (iii) concentration of A:T→T:A mutations at the 5'AG acceptor splice sites, and (iv) unique exon mutational hotspots [10].

Solidifying the mechanistic role of aristolochiaaristolochia in urothelial carcinogenesis has been a remarkable scientific achievement in the past 15 years [10]. It is hoped that with an increasing recognition of the importance of aristolochia, additional measures will be forthcoming to eliminate exposure to this carcinogen in susceptible populations.

Diet

The discussion below focuses specifically on dietary composition and not on inadvertent dietary ingestion of carcinogens discussed elsewhere in the chapter.

Food

Fruit and vegetable intake is likely a protective factor for the development of bladder cancer. Meta-analyses [14, 15] suggest that those with high fruit and vegetable intake have about a 20% lower risk of developing bladder cancer relative to a lower intake. Despite this, numerous individual studies on fruit and vegetable intake have been negative for an association with bladder cancer suggesting that this relationship is far from clear. This protective effect appears to be present when looking specifically at total fruit, total vegetables, cruciferous vegetables, citrus fruits, and yellow-orange vegetables [15]. Other dietary factors associated with increased bladder cancer risk include fried eggs and processed meat [14].

Given the broad macro- and micronutrient composition of individual diets, the mechanisms linking these epidemiologic associations and carcinogenesis are likely multifactorial and incompletely characterized. Possible mechanisms of a protective

effect of dietary composition include antioxidant mechanisms, reduction of carcinogens in meat such as N-nitroso compounds [16], or additional vitamins or other micronutrients which have specific effects.

Fluid Intake

Total fluid intake has been suggested to be a protective factor for bladder cancer, but a conclusive link has been difficult to demonstrate. Mechanistically, total fluid intake could reduce the contact time and concentration of potential carcinogens with the urothelium. There may be a paradoxical effect of fluid intake in those ingesting fluids contaminated with a carcinogen (e.g., arsenic) where a higher fluid intake may result in greater carcinogen exposure. A recent meta-analysis did not find conclusive evidence of a link between fluid intake in bladder cancer, although this was inconsistent across various subgroups and studies [17].

Numerous studies have looked at the specific type of fluid intake and bladder cancer. There may be a signal that green or black tea is associated with a protective effect for bladder cancer, but results are mixed [17, 18]. Most of the research surrounding the protective effect of tea has focused on green tea, where several in vitro studies support a protective effect [19]. The protective effect of green tea is mediated through polyphenols and catechins. These substances may have antioxidant properties or direct induction of apoptosis of bladder cancer cells. Nonetheless, despite this in vitro evidence, clinical studies have not demonstrated a clear protective effect of green tea consumption on bladder cancer [18]. This may relate to the significantly higher concentrations studied experimentally than are likely ingested by green tea drinkers. Associations have also been seen between bladder cancer and milk (protective) and coffee (promoting) [14]. No associations were seen between alcohol intake and sweetened carbonated beverage intake [14]. The mechanisms for these associations are unclear, although components of various beverages have been found in experimental studies to have both pro- and anticancer properties in vitro.

Vitamins

Higher intake of vitamins A, C, D, E, folate, and selenium is associated with a protective effect on bladder cancer risk [14]. The protective effects of these vitamins are likely multifactorial and also likely contribute to the protective effects of the overall dietary composition described above. High-quality clinical studies are still lacking for the use of any vitamin supplement to reduce bladder cancer risk. Further study is essential before promoting any supplementation in clinical practice. This is especially important given prior experiences with prostate cancer, where an increased risk of prostate cancer associated with vitamin E supplementation was only seen after a randomized clinical trial was conducted [20].

Medications

Cyclophosphamide

Cyclophosphamide, a cytotoxic chemotherapy used for a variety of different conditions over the last five decades, has a long-established association with bladder cancer [21]. This is likely a dose-dependent phenomenon with bladder cancer risk being associated with cumulative exposure [22].

The early bladder toxicity associated with cyclophosphamide includes hemorrhagic cystitis. Hemorrhagic cystitis can occur as an acute or delayed event after cyclophosphamide exposure [23]. Acute hemorrhagic cystitis is thought to be mediated by the cyclophosphamide metabolite acrolein. Prevention of hemorrhagic cystitis in patients receiving cyclophosphamide is essential, and effective measures can be taken including diuresis, hydration, continuous bladder irrigation, and MESNA, which interacts with acrolein to form nontoxic products. Delayed hemorrhagic cystitis is often associated with BK polyomavirus, a common cause of hemorrhagic cystitis in immunosuppressed patients [23].

Bladder cancer is a delayed effect of cyclophosphamide treatment, with a median time to development of 7–9 years [23, 24]. Although these cancers are still predominantly urothelial in origin, squamous cell carcinoma and sarcomas are more common after cyclophosphamide exposure. The mechanism of cyclophosphamide-induced bladder cancer has not been clearly elucidated. Acrolein has been found to be mutagenic in vitro although it is not certain if this is the mechanism of bladder carcinogenesis following cyclophosphamide exposure [24]. It is also uncertain how bladder cancer associates with hemorrhagic cystitis and efforts to prevent acrolein toxicity like MESNA use. Alternate mechanisms for cyclophosphamide-induced bladder cancer include other metabolites such as phosphoramide mustard or chloracetaldehyde [25], oxidative stress, and chronic inflammation. Host factors undoubtedly play some role in cyclophosphamide-induced bladder carcinogenesis, with some role seen for both fibroblast growth factor receptor 2 and keratinocyte growth factor receptor in the urothelial response to cyclophosphamide [26, 27].

Pioglitazone

Pioglitazone is a thiazolidinedione used for glycemic control in diabetes. It is an agonist of PPAR-gamma, a transcription factor with higher expression levels associated with bladder cancer cell invasion and migration in vitro [28]. Although some larger studies have been negative for a clinical association between pioglitazone and bladder cancer [29], a meta-analysis of 12 studies supported a 14% increased incidence of bladder cancer in patients treated with pioglitazone [30]. This association appeared to be dose-dependent. In keeping with these data, various regulatory agencies including the United States Food and Drug Administration have issued a warning on the risk of pioglitazone and bladder cancer [31].

Phenacetin

Phenacetin is an anti-inflammatory medication that is no longer in use in most countries. The medication was associated with urinary tract cancers in prior studies. Potential etiologies of its carcinogenic effect include p-aminophenol, a potentially mutagenic metabolite generated within the kidney [32]. Fortunately, the medication has been withdrawn from the United States since 1983, but it may be still used internationally or in adulterants for illicit drugs like cocaine [33, 34].

Radiation

Ionizing radiation is oncogenic in a dose-dependent fashion. Ionizing radiation is thought to induce oncogenesis by free radical formation which results in DNA damage, including direct base alterations or the impediment of DNA strand repair [35]. This process then leads to the activation of oncogenes or inactivation of tumor suppressor genes and subsequent malignancy.

Although an uncommon event, high doses of environmental radiation have a fairly clear link to bladder cancer. Those with significant exposure from the Chernobyl nuclear disaster have been documented to exhibit an increase in urothelial DNA damage, altered DNA damage-repair mechanisms, and urinary bladder lesions [36]. Unsurprisingly, those within highly affected areas seemed to have a significant predisposition towards urothelial carcinoma and dysplasia [36]. Interestingly, a specific alteration in TP53 which is an important driver in high-grade bladder cancer has been found in the bladder cancers of Chernobyl survivors [37].

Contemporary exposure to excessive ionizing radiation more commonly occurs due to cancer treatment. Radiation-based protocols are broadly used across various cancer types. This includes many pelvic cancers where the bladder is in close proximity, such as prostate, rectal, anal, and gynecologic malignancies. This association between therapeutic ionizing radiation and secondary radiation-induced bladder cancer has been understood for decades [38]. The risk of radiation-induced secondary malignancies is undoubtedly influenced by coexisting factors which may predispose a patient to malignancy including environmental factors (e.g., smoking), genetic predisposition, dose, technique, field, and concurrent administration of chemotherapy [39].

Immunosuppression

Immunosuppressed populations are a group at an increased risk for malignancy. The three mechanisms thought to associate immunosuppression and malignancy include a susceptibility to oncogenic viral infections, an impaired ability to eliminate

pathogens which may result in prolonged exposure to inflammation, and a loss of immune surveillance to eliminate cancerous or precancerous cells before they cause harm [40]. In keeping with the critical role of the immune system in carcinogenesis, medications that stimulate the immune system via the PD-1/PD-L1 axis have become standard in treating urothelial bladder cancer [41].

There are several lines of clinical evidence linking immunosuppression and bladder cancer. Firstly, chronic glucocorticoid use was found to be a risk factor for bladder cancer in clinical studies [42]. A recent meta-analysis of 11 studies also supports an incidence ratio of 3.18 for bladder cancer in renal transplant recipients [43]. Although this finding likely relates at least partially to immunosuppression, there is also significant confounding in this population with an increased risk of exposure to other bladder cancer risk factors (e.g., cyclophosphamide for the treatment of immune conditions resulting in the need for a transplant, pioglitazone for the treatment of diabetes resulting in the need for transplant, smoking and atherosclerosis, or aristolochia-induced nephropathy as a cause of renal failure and ultimate need for transplant) [43]. Different causes of immunosuppression likely have different risks of associated bladder cancer—for example, there is no evidence that HIV increases the risk of bladder cancer [44].

Infection

Squamous cell carcinoma caused by *Schistosoma hematobium* infection in Egypt is a prototypical infection-associated cancer. More recently, other infectious etiologies of bladder cancer have been studied and likely contribute to a minority of bladder cancer cases in other settings. In this section, we discuss the postulated infectious etiologies of bladder cancer and their mechanisms of carcinogenesis (Table 2.2).

Recurrent Bacterial Urinary Tract Infections

Recurrent bacterial urinary tract infections have been linked with bladder cancer in some studies [45, 46]. The exact mechanisms for this effect have not been completely characterized. It is known that some bacterial infections produce nitrosamines which may be carcinogenic to the urothelium [47]. These nitrosamines were associated with uropathogens, either produced directly or by the inflammatory response, and had an association with urothelial hyperplasia and neoplasia [47, 48]. Other virulence factors are likely also involved, with additional possible oncogenic mechanisms resulting from the inflammatory response and host factors [49].

Patients prone to urinary tract infections with an especially high risk of bladder cancer include patients with a neurogenic bladder and/or indwelling urinary catheter. Older studies supported a 2.5–10% risk of squamous cell carcinoma of the bladder with a median delay of 17 years from spinal cord injury [50]. The exact

Table 2.2 Putative infectious etiologies of bladder cancer

Infection	Class of pathogen	Susceptible population	Mechanism	Additional comments
E. coli and other uropathogens	Bacterial	Structural or functional abnormalities of urinary tract	Carcinogenic metabolites and inflammatory response	Risk can be mitigated by appropriate urologic management in susceptible patients
Schistosoma haematobium	Parasitic	Africa and Middle East	Direct parasitic actions, immune and regenerative responses, confections, and environmental factors	Population cancer risk can be mitigated by schistosomal eradication
Epstein–Barr virus	Viral	Likely geographic - studies originate from East Asia and Southern Europe	Unclear, although the direct carcinogenic effect of EBV in nasopharyngeal carcinoma and Burkitt's lymphoma has been better characterized	Causative effect on bladder cancer remains unclear
Neisseria gonorrhea	Bacterial	Sexually transmitted infection	Unclear, although inflammatory response has been hypothesized	Causative effect on bladder cancer remains unclear
Human papillomavirus	Viral	Sexually transmitted infection	Direct effect, p53, Rb, and BCL2L1 mediated	Likely a factor in a minority of cases only; potentially modifiable with vaccination
Polyomavirus	Viral	Ubiquitous but more significant with immunosuppression	Possibly direct effect of LTag on p53 and Rb	Causative effect on bladder cancer remains unclear

EBV Epstein-Barr virus

mechanism of this predisposition is likely multifactorial and includes the bacterial mechanisms above, along with significant chronic inflammation associated with the infections, obstruction, and foreign bodies like a catheter [49]. Appropriate urinary tract management in this population has been emphasized more in recent decades, with a greater focus on avoiding indwelling catheters, renal function preservation, urinary stone prevention, urinary tract drainage, and management of infections. Hopefully, as a result of this increasing focus on urinary tract management, more recent studies have found rates of bladder cancer in this population to be lower, with a large series only finding bladder cancer in 0.38% [51].

Schistosoma haematobium

Schistosoma hematobium is a parasitic infection endemic to Africa and the Middle East where it affects an estimated 110 million people [52]. The prevalence of *S. haematobium* in school-aged children is 17.4% overall in Africa and with significant geographic variability [53]. The highest national prevalence estimated in 2012 was in Mozambique at 47.1% [53].

Schistosomal cercariae penetrate human skin after contact in freshwater in endemic areas [54]. In the human host, cercariae transform into schistosomulae and migrate, over several months, to the lungs and then the liver. Maturation occurs in the liver, and *S. haematobium* then migrates to the venous plexi around the bladder and other pelvic organs. Here, they reproduce and shed eggs into the urine for their 3–5 years' lifespan. After the eggs have reached freshwater and hatched into miracidia, they infect snails of the *Bulinus* genus. After 1–2 months of development in the snail host, cercariae are released back into freshwater continuing the cycle of *S. haematobium* infection [54].

Schistosoma haematobium is the best characterized infectious etiology of bladder cancer. This parasitic infection is classified as a Group 1 (carcinogenic to humans) bladder carcinogen by the International Agency for Research on Cancer (IARC) [55]**.** Salem et al. have recently reviewed the changing epidemiology of *S. haematobium* infection during the twentieth century in Egypt [56]. Egypt has classically been the country most associated with *S. haematobium* as Europeans first contracted it and described it here (Salem). The proliferation of the *Bulinus* snail correlated with the construction of dams in the nineteenth and twentieth centuries. The purpose of this construction was to slow water flow and improve irrigation for agricultural purposes. These measures raised the estimated prevalence of *S. haematobium* infection to an estimated 60–70% in parts of Egypt in the early twentieth century. This had significant impacts on the rates of *S. haematobium* sequelae including bladder cancer. In the later part of the twentieth century, an aggressive public health effort involving mass drug administration with praziquantel as well as the eradication of snail populations reduced the prevalence of *S. haematobium* infection to 1.2% in 2006 [56].

The association between *S. haematobium* infection and bladder cancer was first described in the literature by AR Ferguson in a study from Egypt published in 1911 [57]. The predominant histology of bladder cancer in Egypt has historically been squamous cell carcinoma due to the high prevalence of *S. haematobium* infection. A number of theories have been proposed to explain how *S. haematobium* infection causes bladder cancer [58]. Histologically, there is a progression from normal urothelium, to urothelial hyperplasia and squamous metaplasia, towards squamous cell carcinoma or urothelial carcinoma [58]. It is theorized that progression along this pathway relates to a complex interplay between actions of the parasite, the host immune and tissue regenerative response, coinfections, and environmental factors [58]. A recent systematic review and meta-analysis has suggested that, relative to other bladder cancer patients, patients with *S. haematobium* associated bladder

cancer have alterations in ANG, APC, chromosome Y, E-cadherin, sFAS, fibronectin, Oct 3 /4, p15 deletion, p16 deletion, p21 expression, RARBeta2, TERT, and TRAP. Relative to schistosomal patients without bladder carcinoma, a number of genetic alterations are also seen including p53, sFas, glutathione, GST activity, NPip, Npyr, TERT, and TRAP expression [59].

Coincident with public health efforts that have reduced the prevalence of *S. haematobium*, there have been significant reductions in the relative proportion of SCC cases in Egypt [56]. The histology of bladder cancer at major Egyptian cancer centers is now predominantly urothelial carcinoma, similar to North American and European nations. In their series of 1932 bladder cancer patients from the largest hospital in Egypt, Salem and Mahfouz [60] have reported a decrease in the incidence of *S. haematobium* associated with bladder cancer from 80% to 50% (2006–2010 vs. 2001–2005) and a decline in squamous cell carcinoma from 73% to 25% (2006–2010 vs. 2001–2005). In a similar series of 9843 patients treated at the Egyptian National Cancer Institute from 1970 to 2007, there was a decline in *S. haematobium* from 82.4% to 55.3%, an increase in urothelial carcinoma from 16.0% to 65.8%, a decrease in SCC from 75.9% to 28.4%, and an increase in median patient age from 47.4 to 60.5 years [61]. It is likely that these changing trends in bladder cancer in Egypt are regionally influenced, as a 2005 series from South Egypt demonstrated SCC in 67.6% of cases and a history of *S. haematobium* infection in 87.7% [62]. As suggested by the Egyptian story, the elimination of *S. haematobium* with mass drug administration of praziquantel is theoretically achievable. In other endemic regions, *S. haematobium* control efforts are hampered by praziquantel availability and concerns for rapid reinfection given the living situation of the at-risk population [63]. Future efforts will undoubtedly focus on translating our knowledge of *S. haematobium* as a preventable etiology of bladder cancer into public health efforts to eradicate this disease.

Epstein–Barr Virus

Epstein–Barr virus (EBV) is a human herpesvirus strongly implicated in the development of nasopharyngeal carcinoma (NPC)) and Burkitt's lymphoma and Burkitt's lymphoma [64]. There are also postulated links between EBV and other Hodgkin's and non-Hodgkin's lymphomas as well as gastric and other carcinomas [65]. The majority of the human population has been infected with EBV. EBV is transmitted by oropharyngeal and cervical secretions and can be asymptomatic or cause infectious mononucleosis [66].

While the strong association of EBV with various malignancies has long been evident, the direct methods by which this virus promotes oncogenesis are under investigation. Important EBV factors that may have a role in oncogenesis include EB nuclear antigens (EBNA), latent membrane proteins (LMP), and non-coding EB encoded RNA (EBER) [66]. The mechanism for EBV-associated NPC is thought to involve deregulation of cell-cycle checkpoint through p16 inactivation and cyclin

D1 overexpression, intrinsic genetic determinants such as 3p and 9p deletions, and epigenetic modifications associated with a tumorigenic phenotype [67]. In endemic Burkitt's lymphoma, EBV may block apoptosis in B cells with an MYC translocation, promote genetic instability, dysregulate telomere functions, and induce DNA damage to infected cells [68]. Vockerodt et al. have recently reviewed emerging models that explain EBV-induced lymphoproliferative disorders [66].

A series of hypothesis-generating studies have suggested a role for EBV in urothelial cancer. Chuang et al. reported EBV detection rates of 56% in 50 cases of bladder UC and 60% in 10 cases of UTUC. Interestingly, EBV was not detected in any of their 10 controls of normal urothelial tissue [69]. These authors noted that high viral copy number correlated with high-grade disease in stage Ta and T1 disease and that viral copy number was higher in urothelial tissue adjacent to tumor in comparison to areas of normal urothelium. These authors did not note a significant difference in viral copy number in overall patients with high-grade vs. low-grade disease or find an association between EBV status and clinical stage or recurrence-free survival—which the authors attributed to the low numbers in their study. Similarly, Abe et al. found infiltration of EBV-encoded RNA in 26 of 39 (66.7%) bladder cancer specimens compared to 0 of 10 controls [70]. Advanced-stage cancers had a higher prevalence of EBV-positive lymphocyte infiltration (Ta&T1 52% vs. T2–4 92.9%). Three other studies have also supported the presence of EBV in 31–50% of patients with urothelial carcinoma and 0–13.3% of controls.

It is likely that any association between EBV and urinary tract malignancies is specific to urothelial histology. Ng et al. [71] have reported that there was no detection of EBV-encoded RNA or LMP-1 in any of 26 Taiwanese patients with pure squamous cell carcinoma of the upper urinary tract or bladder. Interestingly, this finding contrasts with nasopharyngeal carcinoma which is known to be EBV-associated and is regarded as squamous in origin [71].

It is important to note that studies supporting an association between EBV are predominantly from East Asia and Southern Europe. A strong geographic preponderance for EBV in the pathogenesis of Burkitt's lymphoma and NPC has been noted—and non-endemic forms of these diseases are not as strongly associated with EBV [67, 68]. The association of EBV and UC forms an interesting hypothesis which will undoubtedly be the subject of further investigation in larger and multinational cohorts.

Neisseria gonorrhea

Neisseria gonorrhea is a gram-negative diplococcus that causes a sexually transmitted infection that may be asymptomatic or result in local or systemic symptoms. It is highly prevalent worldwide with an estimated incidence of 106 million cases in 2008, an increase of 2% from 2005 [72]. It is the second most common sexually transmitted infection in the United States with an incidence of more than 600,000 cases annually [73]. Treatment of *N. gonorrhea* is becoming increasingly

challenging as it has progressively developed resistance to a number of antibiotics—most recently the cephalosporins—and previously sulfanilamide in the 1940s, penicillins and tetracyclines in the 1980s, and fluoroquinolones by 2007 [73].

The strongest epidemiologic link between gonorrhea and bladder cancer arises from the Health Professionals Follow-Up Study [74]. This is a prospective cohort that enrolled 51,529 predominantly white men of whom 37,012 could be included in the analysis. A total of 2.9% of men reported a history of gonorrhea; there were 14 bladder cancer cases in these men compared to 272 cancer cases in the control group. Men with a history of gonorrhea had a 1.92-fold (95% CI 1.10–3.33) risk of bladder cancer when controlled for smoking history, race, region of residence, and total fluid intake. Gonorrhea status correlated with increasing severity of bladder cancer—from no association with superficial bladder cancer, weak among less advanced disease (Ta and T1; RR 1.14, 95% CI 0.50–2.59), to stronger in advanced cases (T2-T4; RR 4.07, 95% CI 1.35–12.3). Additional case-control series also support a linkage between a history of gonorrhea and bladder cancer [75, 76]. The validity and mechanism of this epidemiologic association remain under investigation.

Human papillomavirus

Human papillomavirus is a double-stranded DNA virus that infects the majority of sexually active persons in their lifetime [77]. The worldwide prevalence of HPV in women is approximately 10%, although significant variation exists based on geographic location and age [78]. The prevalence in males varies widely from 1.3% to 72.9% in different study populations with risk factors that include HIV infection and sexual behavior [79]. Due to its involvement in a number of well-characterized oncogenic pathways—HPV association with other malignancies is being actively investigated. As discussed by Tolstov et al. in their recent review, HPV may be involved in the pathogenesis of a number of urologic malignancies [80].

Although there are over 100 types of HPV, HPV-16 and HPV-18 are thought to be the most commonly implicated in malignancy [80]. HPV-encoded proteins E6 and E7 have been well characterized and are thought to be the mediators for HPV-induced oncogenesis. E6 and E7 promote the degradation of p53 and Rb, respectively. Both p53 and Rb are tumor suppressor proteins involved in cell cycle regulation—and the p53/Rb pathway is altered in 93% of bladder cancer cases [81]. Mutations in TP53 (gene for p53) and RB1 (gene for Rb) occur in 49% and 13% of bladder cancer cases, respectively—and these are among the most frequently mutated genes in bladder cancer [81]. HPV also may integrate its DNA into the genome of affected cells [82]. This phenomenon was present in one case of bladder cancer in the TCGA analysis where integration of HPV16 DNA into the BCL2L1 gene on chromosome 20—an apoptosis regulating gene—resulted in the overexpression of this gene by a factor of 10. This finding led the TCGA study authors to conclude that viral infection may have a role in the development of some urothelial

carcinomas [81]. Other additional oncogenic mechanisms have been suggested for HPV which are less well characterized [80].

The linkages between HPV and bladder cancer have been investigated for the last three decades [83–85]. A recent meta-analysis by Li et al. identified 52 publications—including 2855 bladder cancer cases—where the HPV prevalence was reported [86]. The overall HPV prevalence was found to be 16.88% (95% CI 15.53–18.31%). The prevalence of HPV in bladder cancer was highest in Asia (24.25%) and lower in North America (13.49%) and Europe (13.11%). High-risk oncogenic HPV subtypes were the most commonly identified subtypes of HPV (15.82% high-risk vs. 1.58% low-risk)—with HPV-16 being the most common subtype identified in 10.81% of cases. When considering 17 case-control studies, there was a 2.84-fold (95% CI 1.39–5.80) increased risk of bladder cancer risk with infection by any type of HPV. When considering the 15 studies reporting data specifically for HPV-16, this odds ratio increased to 5.74 (2.59–12.71).

Since the publication of this meta-analysis, a number of reports have attenuated its enthusiasm for the role of HPV in bladder cancer. A comment on the original Li et al.'s meta-analysis reported reanalyzed data with semi-Bayesian-adjusted estimates which were thought to be more accurate given the wide confidence intervals and heterogeneity of studies included in the meta-analysis [87]. With this adjusted analysis, the original odds ratio was reduced from 2.7 (95% CI 1.4–5.2) to 1.4 (95% CI 1.1–1.8). A number of series have subsequently also reported a very low prevalence of HPV DNA in bladder cancer [81, 88–90].

Some investigators have specifically looked at the role of HPV in the pathogenesis of squamous cell carcinoma of the bladder. Most other HPV-induced cancers are squamous cell carcinomas—which provided the rationale for these studies. Although it has been reported [91], a number of larger studies have suggested that HPV DNA is not routinely present in squamous cell carcinomas of the bladder or urothelial carcinomas with squamous differentiation [91–93]. HPV DNA in primary adenocarcinoma of the bladder has also been reported [91], but is not routinely found [92].

To reconcile the current evidence, the association between HPV and bladder cancer is likely limited to a minority of patients with specific demographics or tumor types. In support of this nuanced approach, Shigehara et al. have reported that HPV DNA was found in 38% (12 of 28) grade 1, 8.5% (6 of 71) grade 2, and 0% (0 of 18) grade 3 bladder carcinomas [91]. The multivariable odds ratio for a patient under 60 having HPV DNA identified was 10.9 (95% CI 2.6–45.3). These findings led the authors to conclude that high-risk HPV is specifically a cause of low-grade bladder carcinomas in younger patients. Similarly, the previously mentioned cases of HPV16 DNA integration into the BCL2L1 gene from the TCGA analysis were supportive of an etiologic role of HPV in urothelial carcinoma—but was only 1 case of 131 high-grade muscle-invasive urothelial carcinomas [81].

An association between HPV and bladder cancer represents a potentially modifiable risk factor for this minority of patients. There is documented effectiveness of an HPV vaccine for both men [94] and women [95]. Within 4 years of the introduction of the HPV vaccine, the prevalence of vaccinated HPV types had declined by

56% in females aged 14–19 in the United States [96]. Despite these declines, only a third of girls aged 13–17 in the United States had received all 3 doses of the HPV vaccine in 2010—with lower rates in Southern states and among the uninsured [97]. Vaccination rates for males are lower than for females, but both seem to be increasing [98]. Vaccination is generally offered to patients under the age of 26—and so it will be several decades before this population might develop bladder cancer. Once this data is available, it may provide us with further insight into linkages between HPV and bladder cancer.

Polyomaviruses

The polyomavirus family comprises 13 double-stranded DNA viruses that, as evidenced by the derivation of their name from the Latin phrase for "multiple tumors," are highly carcinogenic in animals. One member of this family, the Merkel cell polyomavirus (MCV), has also been definitively shown to be carcinogenic in humans [99]. The polyomavirus that is most frequently cited as a potential causative agent for urinary tract tumors is the BK virus (BKV). BKV. infection is thought to be ubiquitous, with seropositivity rates exceeding 90% by mid-childhood [100]. In immunocompetent hosts, acute infection is usually asymptomatic and often leads to a latent infection in renal tubular and urothelial cells wherein active viral replication ceases but the viral genome persists [101]. BKV reactivation leading to active infection is significantly more common among renal transplant patients, producing a cytopathic effect and resultant inflammatory process that causes graft damage in approximately 8% of patients [102].

As is the case with other potentially oncogenic viruses, establishing a causal link between BKV infection and UC is problematic. Given the ubiquitous nature of BKV and the rarity of UC, the classic criteria for establishing an infectious agent as a cause of disease, Koch's postulates, do not apply. Recently, Harald zur Hausen, a pioneer in the field of tumor virology and Nobel laureate, proposed four criteria for establishing a virus as a causative agent in the development of a particular tumor: (1) there should be epidemiologic evidence that infection represents a risk factor for the tumor, (2) genetic material from the virus should be consistently found within tumor cells, (3) transfection of host cells with the viral genome should stimulate cell proliferation in vitro, and (4) induction of tumor proliferation should be shown to depend on a viral gene product [103].

Epidemiologic evidence linking polyomavirus infection to UC is sparse and inconsistent, which is not surprising given that the pervasiveness of these infections makes establishing an association with UC challenging. In a case-control study of 1135 patients with UC and 982 controls, BKV and MCV antibody titers were higher among seropositive cases than seropositive controls, although there was no association between seropositivity itself and UC [104]. Another recent study reported the risk of UC in a cohort of 722 renal transplant recipients to be 11.5% (three of 26 patients) among those with a history of active BKV infection as evidenced by

viruria compared to 2.3% (16 of 696 patients) of those without [105]. Studies examining viral DNA and protein expression within UC tumor cells have been performed almost exclusively in renal transplant patients and have likewise yielded inconsistent results. Multiple case reports and case series have demonstrated immunohistochemical expression of large tumor antigen (LTag), a key factor in the proposed mechanism of BKV-induced oncogenesis, in some but not all bladder and upper tract tumors arising in patients with a history of BK nephropathy [106]. For example, in a recent Taiwanese series, four of seven high-grade invasive bladder tumors expressed LTag [107]. In another study, JC virus (JCV) DNA and LTag were detected in 30 of 33 (91%) and 10 of 33 (30%) UC tumors, respectively, in apparently immunocompetent patients [108]. Nevertheless, large-scale studies comparing the presence of LTag expression and its intensity in malignant tissue versus adjacent normal urothelium are lacking. Finally, although transfection with BKV has been shown to induce proliferation and immortalization of human fibroblasts [109], in vitro transformation of urothelial cells by BKV has, to our knowledge, yet to be demonstrated.

Despite only weak evidence supporting zur Hausen's first three criteria for causality, there exists a well-elucidated mechanism by which polyomaviruses induce tumor development. LTag is known to bind to and functionally inactivate the cell cycle regulatory proteins p53 and pRb, which drives host cells into the S phase and allows the viral DNA genome to be replicated by the host's machinery [110]. Inactivation of these tumor suppressor proteins can also lead to unchecked cell division; not coincidentally, *TP53* and *RB* have been shown to be two of the most frequently mutated genes in high-grade UC [111]. Under normal circumstances, LTag-mediated expression of viral capsid proteins would lead to virion assembly and, ultimately, cell lysis. In this way, active BKV infection would not be able to exert a direct oncogenic effect on host cells. However, Kenan et al. recently reported finding the BKV genome integrated into the genome of UC tumor cells in a renal allograft of a patient with no prior evidence of BK nephropathy [112]. The break in the circular BKV genome that allowed for linearization and subsequent integration occurred in the gene coding for the VP-1 capsid protein, which prevented its expression and, subsequently, viral replication. While the viral gene coding for LTag was left intact, several regulatory regions that normally form part of a negative feedback loop on LTag expression were likewise disrupted, resulting in significantly increased expression of LTag within the tumor cells. This suggests that BKV oncogenicity may be independent of active infection and that its mechanism may be analogous to that of high-risk HPV subtypes, in which viral genome integration into the host cell's DNA results in the disruption of the suppressor protein E2 and results in the overexpression of the oncoproteins E6 and E7 [113]. The authors suggest that BKV integration into the host genome may be a rare and sporadic event, which would account for a weak association between BKV infection and incident UC and the inconsistent expression of LTag in UC tumors.

In summary, while a biologically plausible mechanism for BKV oncogenicity exists, further research is necessary to definitively establish a causative role for BKV in the development of UC. Integration of BKV DNA into the genome of the

host cell may occur only rarely and in the absence of a prior active infection but may play a crucial role in BKV-induced urothelial oncogenesis in the small proportion of tumors in which it does occur.

Smoking

Cigarette Smoking

Tobacco is recognized as one of the most important risk factors for bladder cancer, particularly cigarette smoking. Smoking increases bladder cancer risk by two to four times and is estimated to account for approximately 30–60% of cases [114, 115]. There is a linear increase in risk with intensity and duration of smoking [115–118]; the relative risk is up to five times higher in heavy smokers (defined as >20 cigarettes per day and /or duration >40 years) compared to nonsmokers.

Tobacco is a rich source of over 62 known carcinogenic compounds and reactive oxygen species, particularly aromatic amines such as b-naphthylamine and polycyclic aromatic hydrocarbons (PAHs), as well as N-nitroso compounds [117] (Table 2.3). These compounds are inhaled, absorbed, metabolized, and then partially excreted in the urine. Contact with the upper and lower urinary tracts allow for mutagenesis to occur in the urothelium. These chemicals and their metabolites promote mutagenesis in urothelial DNA by causing single- and double-stranded breaks, base modifications, and bulky adduct formation [117, 119, 120]. Although numerous DNA repair mechanisms exist including the nucleotide excision repair pathways, polymorphisms or mutations in these pathways make some individuals even more susceptible to the carcinogenic effects of smoking [119, 121].

Unsurprisingly, the main intervention that may reduce smoking-induced bladder cancer is smoking cessation. This presumably reduces bladder cancer risk by a reduction in constant carcinogen exposure.

A meta-analysis of 83 studies demonstrated that the relative risk for bladder cancer was 2.04 (95% CI 1.85–2.25) for former smokers, significantly lower than the relative risk of 3.47 for current smokers (95% CI 3.07–3.91) [114]. Duration from smoking cessation matters as well—former smokers who have stopped for 1–3 years have a 2.6 relative risk of bladder cancer compared to only 1.1 (close to baseline) for those who stopped for over 15 years [117, 122].

The mechanism of cigarette smoke exposure is also important in understanding bladder cancer risks. Compared to mouth inhalation only, those who inhale smoke into the throat and chest were at increased risk of bladder cancer [118]. Furthermore, secondhand smoking exposure appears to have a low risk of bladder cancer that is not statistically different compared to nonsmokers, although this finding may not apply to the extremes of exposure [123]. The type of tobacco used also appears to play a role in bladder cancer risk. Unfiltered, high-tar or black tobacco cigarettes, which have a higher concentration of N-nitrosamine and 2-napthyylamine, are associated with a higher bladder cancer risk compared to low-tar or blond tobacco [117,

Table 2.3 Putative carcinogens present in cigarette smoking

Carcinogens	IARC group	Cancer associated with compound
Aromatic amines		Bladder and others
beta-Naphthylamine	1	
4-Aminoiphenyl	1	
2-Toluidine	2A	
N-nitrosamines		Bladder, lung, and others
N-Nitrosonornicotine	1	
4-(Methylnitrosamino)-1-(3-pyridyl)1-butanone	1	
N-Nitrosodiethanolamine	2B	
Polycyclic aromatic hydrocarbons		Bladder, lung, and others
Benzo[a]pyrene	1	
Aldehydes		Lung and others
Formaldehyde	1	
Acetaldehyde	2B	
Phenolic compounds		Others
Catechol	2B	
Caffeic acid	2B	
Volatile hydrocarbons		Lung and others
Benzene	1	
1,3-Butadiene	2A	
Miscellaneous organic compounds		Others
Vinyl chloride	1	
Ethylene oxide	1	
Metals and inorganic compounds		Lung and others
Arsenic	1	
Nickel	1	

IARC International Agency for Research on Cancer

118, 124]. There is also a decreased risk from time since smoking cessation of blond tobacco, but no decreased risk was observed for black tobacco [118].

Electronic Cigarettes

The use of electronic cigarettes or "e-cigarettes" has become increasingly popular, particularly among adolescents and young adults. Introduced in 2007, e-cigarettes rapidly became the most popular form of tobacco used by young people in the United States by 2014 [125, 126]. E-cigarettes consist of a cartridge that holds a liquid solution containing various amounts of chemicals including nicotine, a heating element that is charged by a battery to aerosolize the liquid, and a mouthpiece to facilitate inhalation.

By aerosolizing nicotine, e-cigarettes are marketed as a "safer alternative" to traditional tobacco products by avoiding the byproducts produced by combustion [127]. This is an unproven claim, and it is unknown if risks may even be potentially increased as e-cigarettes contain additives and solvents that can form similar toxic and carcinogenic compounds, such as toxic metal nanoparticles [127]. A recent systematic review of 22 articles describes over 40 different parent compounds and 4 metals found in the urine of e-cigarette users [128]. The majority of e-cigarettes studies have focused on known urinary biomarkers well studied in the tobacco literature including PAHs and volatile organic compounds. Overall, 63 toxicant or carcinogenic metabolic biomarkers were identified, and e-cigarette users were shown to have a higher concentration of urinary biomarkers of several known carcinogenic compounds linked to bladder cancer compared to non-e-cigarette users. This includes pyrene, naphthalene, fluorene, phenanthrene, o-toluidine and 2-naphthylamine [128, 129].

The impact of these findings pertaining to carcinogen exposure with e-cigarette use and how it translates to bladder cancer risk is currently unclear due to their recent introduction and an expected latency period between exposure and cancer development. Further evaluation on the safety of e-cigarettes is warranted due to the rise in popularity and the presence of urinary biomarkers associated with bladder cancer carcinogenesis [130].

Others

Cigar and pipe smokers are thought to have an increased independent risk of bladder cancer, although these populations are difficult to study as they frequently also smoke regular cigarettes [117, 131]. Opium smoking may also increase the risk of bladder cancer; a meta-analysis of 17 studies found an odds ratio of 3.85 (95% CI 3.05–4.87), which was further increased if there was concomitant use of tobacco with an odds ratio of 5.7 (95% CI 1.9–16.3) [132]. Paradoxically, cannabis smoking does not appear to be bladder cancer risk—a cohort study of 34,000 cannabis smokers found an inverse relationship with bladder cancer over an 11-year period [133]. More data will undoubtedly be forthcoming with respect to cannabis and bladder cancer with the recent legalization of cannabis for recreational use in a number of North American jurisdictions.

Gender

Men have a three- to fourfold higher lifetime risk of developing bladder cancer compared to women [134]. This risk is predominantly explained by a higher historical prevalence of smoking and exposure to occupational risks in men [134]. It will be interesting to monitor this observation in the coming decades due to shifting cigarette smoker demographics. Although the prevalence of smoking among men in

the 1950s was higher compared to women, the difference between genders has decreased over time as women became more likely to smoke in the 1970s. Interestingly, the current rate of cigarette smoking is similar between genders, while the bladder cancer incidence is decreasing in men and increasing in women. This undoubtedly reflects changing cigarette smoker demographics in the twentieth century and a several-decade latency period between carcinogenic exposure and bladder cancer development [134].

Although bladder cancer incidence is higher in men, women have more advanced disease at presentation, faster time to recurrence and progression, and as inferior cancer-specific survival [134–136]. These differences are thought to be related to a combination of factors including a delay in diagnosis arising from a misattribution of hematuria and urinary symptoms to urinary infections or menses, health care disparities, differences in smoking and occupational exposure, and potentially anatomic and hormonal factors [136, 137]. Postulated molecular mechanisms for gender differences in bladder cancer include differences in the hepatic metabolic detoxification of carcinogens and differences in the sex steroid hormone pathways [136].

Race

Although African Americans are half as likely to develop bladder cancer as whites, African Americans are known to have a more advanced stage at presentation and poorer cancer-specific survival [134, 135, 138]. Exemplifying this, 5-year bladder cancer-specific survival in white individuals is 82.8% compared to only 70.2% in African Americans [139]. These findings are potentially attributed to health care disparities as a direct biologic link relating to environmental or genetic factors has not been well characterized [139].

Occupational Risk

Occupational exposures have been estimated to account for 5–6% of the attributed risk of bladder cancer [45]. Occupational bladder cancer risk is likely correlated to the degree of exposure to relevant carcinogens occupationally; although numerous occupations are at risk, a recent meta-analysis found the highest relative risk in tobacco workers (RR1.72 95% CI 1.37–2.15) and dye workers (RR13.4, 95% CI1.5–48.2) [140].

The International Agency for Research of Cancer (IARC) has identified many definitive bladder carcinogens to which populations have had historic or current occupational exposure (Table 2.4) [120]. Among the first agents implicated were reported more than a century ago including benzidine and b-naphthylamine that were used in the rubber and dye industries [141]. These aromatic amines cause

Table 2.4 Putative occupationally-related carcinogens and their respective occupations

Occupation	Carcinogen
Aromatic amines	
Textile/dye worker	Benzidine, b-naphthylamine, 4-aminobuphenyl
Leather workers	Benzidine, chlornaphazine
Rubber workers	b-Naphthylamine, 4-aminobiphenyl, benzidine
Newsprinter workers	4-Aminobiphenyl
Hairdresser	4-Aminobiphenyl, 4,4-methylene bis-2-chloroaniline
Firefighters	Benzene, 4-aminobuphenyl, 2-naphthylamine, methoxyaniline, methoxynitroaniline
Polycyclic aromatic hydrocarbons	
Aluminum workers	Benz(a)pyrene, metal worker fluids
Electrical workers	Benz(a)pyrene, cadmium, antimony, arsenic
Mechanics	Naphthalene, acenaphthene, phenanthrene, mineral oils
Coal workers	Pyrene, naphthalene
Truck drivers	Naphthalene, benzidine, acenaphthene, phenanthrene, diesel exhaust, 4,4-methylene bis-2-chloroaniline

carcinogenesis by bind to and directly damage DNA [142]. Textile workers are similarly at increased risk from contact with pigments, dyes, and synthetic materials, specifically b-naphthylamine, benzidine, 4-aminobiphenyl, and nitrobiphyenyl. In the 1970s and 1980s, newspaper presses used mineral oils pigmented with carbon black, benzidine, and induline/nigrosin dyes, exposing workers to 4-aminodiphenyl. The current list of suspect occupationally related carcinogens associated with bladder cancer risk includes aromatic amines, particularly as benzidine and b-naphthylamine [117], as well as 4-aminobiphenyl, ortho-toluene, 4,4′-methylenebis (2-choloaniline), metal working fluids, polyaromatic hydrocarbons (PAH), tetrachloroethylene, and diesel exhaust [140]. A discussion of selected specific occupational toxins is included below.

Polyaromatic Hydrocarbons

An increased bladder cancer risk is associated with polyaromatic hydrocarbon (PAH) exposure. These chemical compounds contain only carbon and hydrogen and have multiple aromatic ring structures. The IARC classifies PAHs as possible or indeterminate carcinogens that occur naturally in coal, oil deposits, and gasoline and are also produced by thermal decomposition of organic matter including tobacco. Specific PAHs include benzo(a)pyrene, naphthalene, fluorene and phenanthrene [117, 143]. PAHs have been found to be mutagenic. After being metabolized, PAHs produce mutagenic metabolites including diol epoxides, quinones, and

radical PAH cations [121]. These metabolites bind DNA and form bulky DNA adduct complexes, leading to replication errors or base errors that result in oncogenesis.

Occupationally-related bladder cancer mortality is highest in occupations with likely PAH exposures including metals, aluminum, glass, and electrical workers [140]. Workers are exposed to PAHs through combustion and diesel fumes, metal working fluids and coal tar products. High PAH exposure occurs during aluminum manufacture where coal tar and pitch anodes evaporate at time of electrolysis to produce benzo(a)pyrene vapor. Aluminum production workers have a 30% increased incidence of bladder cancer which increases with cumulative exposure [140]. In machinists, exposure to mineral oils (used as metal-working fluids in the cooling, lubricating, and cutting of metals), fumes, solvents, paints, and greases increases the risk of bladder cancer. Mineral oils are known carcinogens due to the high PAH content, and bladder cancer risk increases with the intensity, duration, and cumulative exposure, as well as the type of metal working fluid: straight (high risk) compared to soluble and/or synthetic fluids (low risk) [144]. Truck drivers, miners, and marine workers develop bladder cancer risk from inhalation of diesel exhaust fumes that contain PAHs and other chemicals with mutagenic effects in a dose-dependent fashion [145]. Furthermore, the infrequent voiding habits of these workers may increase the contact time of carcinogens to the urothelium.

The interaction between oncogenesis and PAH exposure is likely complex, as some occupations with known PAH exposure including coke production and coal tar and carbon-electrode manufacture have not shown to lead to an increased bladder cancer risk [143]. A pooled analysis of European case-control studies reported a combined relative risk of 1.23 (95% CI 1.07–1.5) for high exposure to PAHs, and 1.27 (95% CI 1.04–1.54) for high exposure to benzo(a)pyrene, and a 15% increased risk of bladder cancer for high exposure to diesel engine exhaust [146].

Hair Dye

Hair dyes vary greatly in their chemical formulation and exposure by inhalation or skin contact. Prior to the 1970s, hair dyes contained mutagenic compounds similar to the aromatic amines used in the industrial setting, namely, 4-aminobiphenyl [147]. In the late 1970s, some of these chemicals have been shown to cause cancer in lab animals leading to hair dye manufacturers to change the chemical components of their products. Since the 1970s, 4-aminobiphenyl has been restricted leading bladder cancer risk to decrease from 3–9 times to 1.2–1.3 times [142]. A review of over 81,000 occupationally exposed individuals such as hairdressers, stylists, and barbers, from 10 cohort studies estimate a relative risk of 1.4 [148]. Thus, the IARC has classified workplace exposure as a hairdresser or barber as “probably carcinogenic to humans” [148]. Although workplace exposure is likely important,

individuals who have had their hair dyed have not been shown to have a consistent increase in bladder cancer risk. A population-based case-control study no relation between personal hair dye use and bladder cancer, and the estimated risk was very close to 1 [147]. A meta-analysis of ten studies of personal hair dye use found a pooled RR of 1.01 (95% CI 0.89–1.14) for bladder cancer, which was consistent irrespective of study design and gender [149].

Genetic Predisposition

Genetic Polymorphisms

In a recent meta-analysis of 154 studies, a number of polymorphisms were identified to have a bladder cancer association (Table 2.5) [150]. Statistically significant associations included genes associated with detoxification (GSTM1, NAT2, CLK3, UGT1A8), nucleotide excision and repair (ERCC2, XPC), homologous recombination (NBN), cell cycle regulation (CCNE1, MYC), and others (ACTRT3, TERT, TP63, JAG1, TMEM129, CWC27, NR, CDKAL1, JRK, LSP, SLC14A1, SLC14A2, C20orf187, CBX6) [150]. These polymorphisms have been identified through large-scale genome-wide association studies and the specific causative effect or functional implication of a polymorphism can sometimes be difficult to characterize. Nonetheless, the functional importance of certain molecular pathways in carcinogenesis suggests the importance of further characterizing some of these associations.

Germline Mutations

A minority of patients with bladder cancer have pathogenic alterations in cancer-predisposing genes present in the germline (Table 2.5). This group of patients is more common than previously thought, a finding highlighted by increasing accessibility of genetic sequencing. One series of 1038 patients with urothelial carcinoma found a 24% rate of pathogenic germline variants. The majority of these pathogenic germline variants were in DNA damage repair genes (78%) with MSH2 (3.5%) and BRCA1/2 (4.4%) having the highest frequency [151]. Another cohort of 586 patients with urothelial carcinoma identified a 14% rate of pathogenic or likely pathogenic germline variants. The most commonly altered genes included BRCA2 (1.5%), MSH2 (1.4%), and BRCA1 (1.4%) [152].

Mechanistically, germline alterations in DNA repair genes are cancer-promoting due to their tumor suppressor activity. Mismatch repair genes, such as MSH2 and MLH1, correct mismatched nucleotides in paired DNA strands arising from DNA replication errors and recombination [153]. These genes may also play a role in

Table 2.5 Suspected genetic causes of bladder cancer

Genetic factor	Mechanism
Cancer-predisposing genes	
BRCA1	Double-strand DNA break repair
BRCA2	Regulate homologous recombination
MSH2	Mismatch repair
Polymorphisms	
GSTM1, NAT2, CLK3, UGT1A8	Detoxification
ERCC2, XPC	Nucleotide excision and repair
NBN	Homologous recombination
CCNE1, MYC	Cell-cycle regulation
ACTRT3, TERT, TP63, JAG1, TMEM129, CWC27, NR, CDKAL1, JRK, LSP, SLC14A1, SLC14A2, C20orf187, CBX6	Other mechanisms

suppressing homologous recombination and DNA damage signaling [153]. Clinically, these patients have hereditary nonpolyposis colorectal cancer (HNPCC)) or Lynch syndrome or Lynch syndrome—a cancer-predisposing syndrome that includes urothelial carcinoma along with colorectal, endometrial, biliary, small bowel, ovarian, and sebaceous adenoma of the skin [154]. Meanwhile, BRCA1 has a role in repairing double-stranded DNA breaks along with other regulatory functions [151]. BRCA2 regulates homologous recombination and mediates recruitment of recombinase RAD51 to DNA double-stranded breaks [151]. Both BRCA1 and BRCA2 predispose to a hereditary breast and ovarian cancer syndrome. These syndromes, especially BRCA2, are increasingly recognized to have other associations such as prostate and pancreatic cancers [155].

Conclusion

Molecular biology has significantly advanced our understanding of the critical role of a number of environmental and genetic factors in bladder carcinogenesis. This understanding of the fundamentals of bladder cancer provides an opportunity to have significant clinical impact in the prevention, screening, or treatment of bladder cancer. Some of this knowledge has already translated into action, such as *S. haematobium* eradication in Egypt [56], risk factors being incorporated into the diagnostic algorithm for microhematuria [156], or immunotherapy being used as treatment for bladder cancer [41]. Public health measures to reduce carcinogen exposure combined with a better understanding of how to modulate host factors involved in carcinogenesis will reduce the global burden of bladder cancer in the coming decades.

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Chapter 3
Strategies for Bladder Cancer Screening

Lauren Folgosa Cooley and Joshua J. Meeks

Introduction

Bladder cancer (BC) is the sixth most common cancer in the United States (US) with an estimated 81,400 new cases and 17,980 attributable deaths in 2020 alone [1]. A diagnosis of BC is made after symptoms (e.g., gross hematuria) or diagnostic tests (e.g., urinalysis) identify signs of malignancy. To date, screening for BC in asymptomatic adults, even if high-risk, is not recommended by the US Preventive Services Task Force (USPSTF) with a Grade I designation (insufficient evidence to assess benefits and harms of screening) [2]. It may be possible to [1] identify a high-risk group of patients and [2] perform a low-cost but highly sensitive screening test to identify patients at risk for BC. The potential benefit of BC screening is to identify cancer in an asymptomatic patient at an earlier stage, thus decreasing mortality and potentially decreasing the morbidity of cancer treatment associated with higher-stage BC. Herein, we will discuss the rationale and potential strategies for BC screening in high-risk, asymptomatic patients including past and future BC screening trials.

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Rationale for Screening

Despite significant improvements in survival in most solid tumors including BC, ~25% of patients will have locally advanced (muscle-invasive), and ~10–15% will have metastatic disease at the time of diagnosis [3, 4]. Patients who present with muscle-invasive or metastatic BC have a much shorter cancer-specific survival (CSS) compared to patients presenting with non-muscle-invasive disease (median CSS HGT1 >10 years vs T2 ~5 years) [5–8]. A recent effort to specifically characterize overall (OS) and cancer-specific (CSS) survival among BC patients by tumor stage (T-stage) was performed using two large US hospital and population-based cohorts, the National Cancer Database (NCDB) and the National Cancer Institute Surveillance, Epidemiology, and End Results database (SEER), respectively [8]. Therein, each increasing T stage from LGTa to T4 disease conferred a significantly worse OS and CSS. Important to the rationale for screening, there was a significant difference between T1 and T2 stages for both OS and CSS (SEER OS - T1HG: HR 1.68, 95% confidence interval (CI) 1.63–1.73 vs. T2: HR 3.39, CI 3.30–3.49, $p < 0.001$) (SEER CSS-T1HG: 72% 10 year-CSS, HR 4.24, CI 4.01–4.47 vs. T2: 48% 10 year-CSS, HR 12.18, CI 11.57–12.82, $p < 0.001$) [8]. Therefore, an earlier stage at diagnosis (≤T1) could potentially improve survival and serve as a rationale for further investigation of targeted screening in BC.

Furthermore, the morbidity and cost of treatment are important factors to consider for BC screening. Depending on the stage at diagnosis, treatment options vary greatly. Earlier-stage diagnosis (non-muscle-invasive disease) is associated with more treatment options, which are often less morbid and less costly and allow for bladder preservation [9, 10]. The more advanced stage at diagnosis (≥T2) often carries an increased cost of treatment, toxicity related to systemic chemotherapy, and morbidity associated with cystectomy or trimodal therapy [7, 9, 10].

High-Risk Populations

There are several clinical, demographic, and environmental factors that have been associated with an increased incidence of BC (see Chap. 2). Some of these factors include smoking history, industrial chemical and metal exposures (e.g., aromatic amine exposure in the dye, paint, and rubber industry), Lynch syndrome, family history of bladder cancer, demographic factors such as older age and Caucasian race, and microscopic hematuria [11–20]. Due to the low overall prevalence of BC, screening a non-risk stratified population would be unlikely to yield benefit and would be cost-prohibitive. Alternatively, identifying a higher-risk population of patients would focus screening efforts on patients most likely to benefit. Two risk factors for BC that may identify patients for screening include age and significant smoking history. These factors are among those recently identified by the most recently updated of the American Urological Association Guidelines on

microscopic hematuria to be critical for determining the risk of urothelial carcinoma and thus patient workup recommendations [21]. Similarly, age, smoking history, and the presence and degree of microscopic hematuria will likely be most meaningful in selecting a BC screening cohort.

Age

The mean age of patients in the United States diagnosed with BC is 73 years with 90% of patients being over 55 years old [1]. Given the need to identify cancer at the earliest possible stage, prior screening trials have typically utilized between ages 50–60 years as their starting age for BC screening [22, 23]. However, given the heterogeneity in study design amongst available screening trials (discussed below), it is still difficult to discern the precise age to initiate screening. When comparing to lung cancer with a mean age at diagnosis of 70 years, the National Lung Cancer Screening Trial included patients 55–74 years, and the USPSTF ultimately recommended annual low-dose computed tomography in adults 50–80 years for eligible patients based on smoking history and life expectancy [24–26].

Cigarette Smoking

Former or current cigarette smoking triples the relative risk of being diagnosed with BC compared to nonsmokers [12]. While smoking habits have changed, the population attributable risk of BC for cigarette smoking has been estimated at ~50–65% in men and ~20–30% in women [12, 27, 28]. In a National Institute of Health Diet and Health survey study from 1995 to 2006, Freedman et al. found that former (119.8 per 100,000 person-years; HR 2.22 (CI 2.03–2.44)) and current (177.3 per 100,000 person-years, HR 4.06 (CI 3.66–4.50)) smokers had a significantly higher incidence of BC compared to never smokers (39.8 per 100,000 person-years) [12]. The incident rates were even higher than estimated in historic studies (summary risk estimate of current smoking from 1963 to 1987, HR 2.94 (CI 2.45–3.54)) potentially reflecting the consequences of changing cigarette composition over time (e.g., increase in nitrosamines and other carcinogens) [12]. Krabbe et al. found that men older than 60 years with a smoking history of >30 pack years were at the highest risk for BC (rate 2/1000 persons) using the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO) and National Lung Cancer Screening Trial (NLST) cohort [13]. Current and former smokers with a 30–50 pack year (PY) or >50 PY smoking history had a significantly higher incidence than never smokers; but, current smokers carried the highest risk (compared to never smokers, 30–50 PY prior smoker HR 2.904, 30–50 PY current smoker HR 3.693, >50 PY prior smoker HR 3.722, >50 PY current smoker HR 4.343) [13]. These findings speak to the continued importance of smoking cessation counseling to reduce the incidence of

BC. Since the amount of smoking (PY) is decreasing, it is unclear whether the number of years of smoking confers a similar risk to the number of cigarettes smoked.

Prior Screening Trials: Comparing Cohorts and Methods of Screening

Urine Dipstick (Chemical Reagent Strip for Hemoglobin)

Due to the low prevalence of BC, the ideal screening procedure must have a high diagnostic sensitivity. Thus, prior screening trials have used a urine dipstick, or a chemical reagent strip that tests for hemoglobin, as the primary method of screening asymptomatic adults [22, 23, 29, 30]. The presence of hematuria on a urine dipstick has ~52% sensitivity and 82% specificity for detecting urothelial carcinoma (UC) [31]. Additionally, the dipstick is noninvasive and of low initial cost [32]. Britton et al. found that the rate of dipstick hematuria in asymptomatic men ($n = 578$) aged 60–85 was 13% (single test) and 9% (multiple tests positive over 10 week period) [23]. Of these men, 4 (4.6% of men with dipstick hematuria) were diagnosed with BC and 41 (47%) with other urologic diseases (Table 3.1) [23]. In 1995, Messing et al. evaluated 1575 asymptomatic men aged ≥50 years who underwent home urine dipstick screening for BC compared to age-matched men diagnosed with BC in the Wisconsin cancer registry (unscreened) [29]. Overall, 21 BC cases were detected by screening (21/258 men with hematuria, 8.1% BC detection rate). While rates of detection of low-grade Ta or T1 BC did not differ in screen vs unscreened men (52.4% vs 56.8%, $p > 0.20$), MIBC was significantly higher in unscreened men (4.8% vs 23.9%, $p = 0.007$). Unscreened men were significantly more likely to die of BC within 2 years of diagnosis compared to screened men with follow-up times ranging from 30 to 102 months (16.4% vs 0%, $p = 0.025$) [29]. A subsequent update at 14 years of follow-up again revealed no screened men had died of BC compared to 20.4% of unscreened men ($p = 0.02$) (Table 3.1) [22].

Urine Cytology

Urine cytology is the cytopathologic evaluation of a voided urine specimen or bladder washing collected at the time of cystoscopy for the presence of atypical or malignant urothelial cells. If performed at the time of cystoscopy, there is an estimated cost of $400 (utilizing Medicare cost analysis from 2002) [33]. A meta-analysis of available urine biomarkers found that urine cytology had a 34% sensitivity and 99% specificity for the detection of UC, but median sensitivity significantly increased with increasing tumor grade (median sensitivity (95% CI) by grade: Ta 0.15 (0.09–0.25), T1 0.46 (0.34–0.59), and ≥T2 0.55 (0.35–0.73)) [31].

Table 3.1 Overview of bladder cancer screening trials

Study	Number of patients screened	Targeted population	Method of screening	BC detection in screened population[a]	Outcomes
	n			*n* (%)	
Britton et al. 1989	578	Men, aged 60–85 years	Urine dipstick	4/61 (6.6%)	Prevalence of dipstick hematuria (132/578, 23%)
Britton et al. 1992	2356	Men, aged ≥60 years	Urine dipstick + urine cytology	17/319 (5.3%)	Prevalence of dipstick hematuria (474/2356, 20%) Of those with BC (17, 5.3%), 10 (58.8%) had abnormal urine cytology
Theriault et al. 1990	Not reported	Aluminum workers, aged ≤65 years	Urine cytology	79	No change in CSS with screening (OR 1.01, 95% CI 0.34–3.04) Screening led to modest increase in detection of earlier stage BC (67% prior to screening vs 77% post screening; $p > 0.1$)
Messing et al. 1995	1575	Men, aged ≥50 years	Urine dipstick	21/258 (8.1%)	Detection rate of ta or T1 BC (52.4% screening vs 56.8% unscreened, $p > 0.20$) Detection rate of MIBC (4.8% screened vs 23.9% unscreened, $p = 0.007$) BC specific mortality within 2 years (0% screened vs 16.4% unscreened, $p = 0.025$)
Messing et al. 2006	1575	Men, aged ≥50 years	Urine dipstick	21/258 (8.1%)	Update to Messing et al. 1995 BC specific mortality (0% screened vs 20.4% unscreened, $p = 0.02$)

(continued)

Table 3.1 (continued)

Study	Number of patients screened	Targeted population	Method of screening	BC detection in screened population[a]	Outcomes
	n			*n* (%)	
Lotan et al. 2009	1502	Men and women, aged ≥50 years, ≥10-year smoking history, ≥15 years high-risk occupational exposure	BladderChek (NMP22)	2/69 (2.9%)	Low cancer detection rate

[a]Bladder cancer detection in screened population who also agreed to cystoscopy evaluation
BC bladder cancer, *CSS* cancer-specific survival, *CI* confidence interval, *NMP22* nuclear matrix protein 22

Thus, it may not be the ideal tool for screening. In a general population of men over 60 years, Britton et al. found that the addition of urine cytology to dipstick hematuria may be a more effective screening strategy than dipstick alone [30]. Of the 2356 screened asymptomatic men over the age of 60 years, 474 men (20%) had dipstick hematuria of which 5.3% (17/319 who agreed to further evaluation) had asymptomatic BC. Of these 17 men, 10 (58.8%) had abnormal urine cytology suggesting that combining dipstick with urine cytology may be more accurate in predicting BC in a general population (i.e., low- and high-risk men) (Table 3.1) [30]. When applying urine cytology screening to a high-risk cohort, specifically aluminum factory workers in Quebec, screening led to an increase in detection of earlier stage BC although not significant (67% prior to screening vs 77% post-screening implementation, $p = 0.1$) [34] with no improvement of cancer-specific survival with screening (OR 1.01, 95% CI 0.34–3.04) (Table 3.1) [34]. However, it remains to be determined that application of urine cytology with or without urine dipstick screening to other high-risk populations such as former or current cigarette smokers would impact the stage of diagnosis or cancer-specific survival.

Other Urinary Biomarkers

There are many other urinary biomarkers available with ranges of sensitivities and specificities in detecting UC. Many of these, such as ImmunoCyt and UroVysion, are approved by the Federal Drug Administration for the diagnosis or monitoring of BC rather than screening but may have implications in future screening trials. While there is a chapter herein dedicated specifically to urinary biomarkers (Chap. 11), we will discuss Nuclear Matrix Protein 22 (NMP22) BladderChek (Matritech, Newton, MA) and its utilization in BC screening. NMP22 is a nuclear mitotic apparatus protein which is released upon urothelial cell death [35, 36]. NMP22 is substantially

elevated in patients with BC, but may also be increased in benign conditions such as urinary tract infections, stones, or hematuria [35, 36]. BladderChek is a lateral flow immunochromatographic assay able to be performed as a point of care test for an estimated cost of $24 [10]. In a study of BC recurrence, BladderChek was able to increase the detection of BC significantly above the ability cystoscopy alone (99.0% vs 91.3%, $p = 0.005$) with a sensitivity and specificity of 49.5% and 87.3%, respectively [35]. Lotan et al. subsequently utilized BladderChek in a BC screening trial of high-risk patients (all age >50 years with 10-year or greater smoking history or 15-year or more high-risk occupational exposure) [37]. Of 1175 men and 327 women, 85 participants (5.7%) had a positive BladderChek of which only 2 cancerous lesions were identified (1 low-grade Ta and 1 multifocal, high-grade Ta) (Table 3.1) [37]. Despite targeting a more high-risk cohort, cancer detection was very low highlighting challenges in BC screening including BC prevalence among even high-risk cohorts and detection rates needed to rationalize screening costs.

Looking Forward: Design of and Challenges in an Ongoing Bladder Cancer Screening Trial

There are many challenges to designing a prospective BC screening trial including (1) identification of the optimal cohort to screen, (2) identification of the incidence of BC in a screened population (sample size calculation), (3) determination of the correct endpoint to evaluate the effectiveness of screening, and (4) selecting the optimal control cohort to name a few. In the future, we hope to apply these restraints in an at-risk cohort to determine if screening can improve the survival of patients with screen-detected BC.

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Chapter 4
Staging of Bladder Cancer

Rathika R. Ramkumar and Samuel C. Haywood

Introduction

After establishing a diagnosis of bladder cancer, practitioners must stage cancer in order to guide treatment. Staging is divided into pathologic and clinical staging. As the name suggests, pathologic staging relies on tumor histopathology from surgery or biopsy while clinical staging considers pre-surgical imaging and physical examination. Accurate staging guides both treatment decisions and prognostic discussions for patients. This is imperative in bladder cancer as staging may determine the need for radical cystectomy versus a bladder-sparing option. This chapter reviews staging considerations for bladder cancer from initial diagnosis to surveillance protocols.

Diagnosis

A variety of tools exist for diagnosing bladder cancer including cystoscopy, urine cytology, and transurethral resection of bladder tumor (TURBT)). Cystoscopy is the mainstay in the workup of suspected bladder cancer. While it is more invasive than a voided urine test, flexible cystoscopy is frequently and easily done in the office. Urine cytology can be used on its own or as an adjunct to cystoscopy. A positive urine cytology signals malignancy anywhere along the urinary tract, and in the

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absence of upper tract pathology or grossly visible bladder tumor, the patient requires bladder mapping biopsies and prostatic urethral biopsy [1]. A negative urine cytology, however, does not exclude bladder cancer. Urine cytology is more sensitive for higher-grade tumors (84% for HG versus 16% for LG tumors), while detection of carcinoma in situ (CIS)) is variable (82–100%) [2, 3]. If office cystoscopy demonstrates a bladder tumor, the patient should proceed to the operating room for TURBT or cold-cup biopsy to obtain pathologic diagnosis.

Urinary biomarkers are less established in this diagnostic paradigm (see Chap. 11). The most studied in bladder cancer is a nuclear matrix protein, NMP-22, levels of which are up to 20 times higher in malignant cells than normal cells [4]. A commercial ELISA is available that quantifies NMP-22 levels in the urine. Sensitivities for NMP-22 range from 69% to 90% and roughly 75% with CIS [4]. Other urinary biomarkers exist as well, and while most have increased sensitivity to bladder cancer when compared to urine cytology, it is at the cost of lower specificity [1, 2]. Ultimately, no urine test can replace cystoscopy for initial visual diagnosis.

Staging Modalities

TURBT

The gold standard for diagnosis of bladder cancer is with transurethral resection of bladder tumor (TURBT). TURBT is diagnostic, and in the case of non-muscle-invasive bladder cancer (NMIBC), it can be therapeutic as well. Optimizing staging accuracy is largely dependent on providing the best specimen for pathologists and performing a bimanual exam to assess for the palpable or "fixed" local extent of disease. A full discussion of the TURBT is beyond the scope of this chapter, but here, we will focus on particular variables (cystoscopy enhancements, resection methods) that may influence staging.

Cystoscopy Enhancements

Standard cystoscopy uses white light to identify tumors, and this can be enhanced with the use of blue-light (BLC) or narrowband imaging (NBI). Each has its own advantages and disadvantages as reflected in Table 4.1 (see also Chap. 9). Most tumors are visualized with white light cystoscopy (WLC) although smaller tumors and CIS can be missed. Also referred to as Cysview, or fluorescence cystoscopy, BLC relies on the biochemical synthesis of photoactive porphyrins (PAPs) from intravesically instilled hexaminolevulinate hydrochloride (HAL). These PAPs preferentially accumulate in mitotically active cells like in bladder cancer and can be seen as red or pink under blue light (wavelengths 360–450 nm). In contrast,

Table 4.1 Different types of cystoscopy used in the detection of bladder tumors

	Normal tissue appearance	Tumor color	Advantages	Disadvantages
White light cystoscopy	Pink or white (normal tissue)	Variable	Standard cystoscope No additional filter required No intravesical instillations	Difficult to appreciate CIS Smaller tumors may go unnoticed
Fluorescence cystoscopy	Blue	Red or pink	Improved identification of tumor	Requires pre-procedure intravesical HAL instillation Needs appropriate timing with the start of the case Need BLC hardware/ capability
Narrowband imaging	White	Brown or green	Outlines tumor well	Need NBI hardware/ capability

NBI relies on image enhancement rather than a biochemical pathway. It employs two specific bandwidths, blue (415 nm) and green (540 nm), that are strongly absorbed by hemoglobin in superficial tissues. As a result, highly vascularized tissues, like cancer cells, will absorb these wavelengths more and appear distinct from surrounding, less vascularized normal tissue. Compared with stand-alone WLC, the concomitant use of BLC or NBI has been shown to have higher tumor detection rates on initial TURBT [5, 6], and their use can reduce recurrence rates [7].

Resection Method

The method of resection is just as important as identifying areas to resect. There is no standardized approach, but most resect any exophytic component followed by the resection of the base and circumferential margins of up to 1 cm [8, 9]. Separately sending resection tissue as superficial and deep specimen can help better define the muscle layer. There are two methods of resection: conventional or *en bloc*. In the conventional approach, all visible tumor is resected piece by piece with loop electrocautery until detrusor fibers are seen to ensure muscle is captured in the specimen. This method splits the tumor as resection is underway. While this is the most widely used approach, it is not without its faults. Conventional TURBT (cTURBT)) works directly against the basic oncologic principle to not break up the tumor. Fragmented tumor risks seeding malignant cells for reimplantation in the bladder. The greatest criticism of conventional TURBT, however, is the high percentage of understaging. An absence of detrusor muscle (DM) in a submitted tumor is the main

Table 4.2 Characteristics of tumor most feasible for EBR according to EAU consensus statement

Tumor characteristic	Consensus
Size	≤3 cm
Quantity	<4 tumors
Location	Any, caution at dome

reason for this with studies reporting up to 56% of tumors without DM [10]. Understaging has been shown to lead to worse recurrence-free survival (RFS) rates in patients with NMIBC, and it risks inadequate treatment of muscle-invasive disease. The importance of a "second look" cannot be overstated for accurate staging, and will be discussed further in the chapter.

In contrast to cTURBT, *en bloc* resection (EBR) is a newer surgical technique with the goal of removing the bladder tumor in one piece without compromising tissue quality or safety. EBR has been found to greatly increase the likelihood of detrusor muscle (DM) being present with studies reporting 97–100% of DM) in specimen [11, 12]. Multiple studies have found EBR to have lower risks of bladder perforation, less operative time, and possibly less chance of tumor seeding and reimplantation due to resection of the tumor as a whole [9, 11, 12]. However, this method is not always practical, and cases must be appropriately selected (Table 4.2). Due to the increasing interest globally in EBR, the European Association of Urology (EAU) devised an International Consensus statement [9]. Tumor size can be the major limitation, with 3 cm being the cutoff in most studies. A modified approach can still be done for tumors above this cutoff. Also, if *en bloc* resection is successfully performed but the specimen is too large to be removed from the bladder as one piece, the tumor can be divided into a few pieces. Surgeons can feasibly do EBR for multiple sites though operative time would increase depending on the total number of tumors. As always, bladder dome tumors are more technically difficult to resect, regardless of resection method, and should be done cautiously. Some tips for successful resection include making a circumferential marking around the tumor before resection, ensuring the mark is at least 5 mm from any other tumor, and incising at the level of the detrusor muscle. If using a laser, a fan-shaped incision can be made through the bladder wall to expose the base. Surgeons can then take advantage of the resulting hydrodissection lifting the incised tumor to aid in resection [12]. Also, additional biopsies or resection of the tumor base is not necessary if EBR is done correctly since muscle should be included with the initial specimen [9].

Conventional TURBT remains the mainstay for remains the mainstay for diagnosis of bladder cancer. It has the advantage of being able to resect irrespective of tumor size. EBR is a feasible and safe alternative that can provide improved specimen quality with higher rates of DM. Smaller and fewer tumors and those farther from the dome are more advantageous for EBR. Bladder tumors that do not fit these parameters can still be resected via a modified approach to optimize DM in the specimen.

Influence of Energy Type

There are two main energy sources for TURBT: electric and laser (Table 4.3). Conventional TURBT uses electrical energy (monopolar or bipolar), while *en bloc* resection can be done with either type. There is not agreement in the literature on whether monopolar or bipolar energy has greater DM rates in specimen. In a prospective, randomized controlled trial (RCT) by Teoh et al., bipolar TURBT had superior DM sampling compared with monopolar TURBT (84.6 vs 67.7, $p = 0.025$) [13]. An earlier RCT by Venaktramani et al. did not find a difference [14]. There is consensus though that thermal injury is problematic with electrocautery. It can damage surrounding normal tissues and render tumor specimen poor quality. Carbonization effects from monopolar energy cause resected tumor to adhere to the loop yielding significant artifact from charred tumor [15]. In one series, cautery artifact (monopolar or bipolar) was found to understage urothelial cancer from initial TURBT by up to 6% for large tumors [16]. This is an inherent risk with electrical energy though it may be less with bipolar [14, 15, 17]. Aside from cautery artifact, incomplete resection is another cause for understaging with cTURBT. Electrocautery was initially thought to be the reason for this; however, monopolar energy has been used successfully with EBR to render whole specimen with high rates of DM present. Initial studies used a modified loop, usually a J-shaped electrode fashioned from a loop [11, 18]. More recent studies have shown feasibility of monopolar and bipolar EBR without modification of the conventional loop electrode [11, 19]. These studies report near 100% of DM in EBR specimen compared to lower percentages (54%) with cTURBT [19].

Laser energy can also be used to treat bladder tumors. Laser vaporization has been shown to be both a safe and feasible alternative to cTURBT for treating NMIBC [15, 20]. Successful *en bloc* resection has been performed with holmium, thulium, and green light lasers. The high-power tissue vaporization potential of these lasers

Table 4.3 Different energy modalities and their use in bladder tumor resection methods

Energy type	Energy source	Resection method
Monopolar	Electrocautery	cTURBT or EBR
Bipolar	Electrocautery	cTURBT or EBR
Hydrodissection	Electrocautery/ waterjet	EBR
Holmium (Ho: YAG)[a]	Laser	EBR
Thulium (Tm: YAG)[a]	Laser	EBR
Potassium-titanyl-phosphate (KTP: YAG), also known as green light	Laser	EBR

[a]Holmium and thulium energy are the more commonly used lasers for EBR

allows for excellent hemostasis. Their use for treatment of bladder cancer, however, was initially limited given the concern that vaporization does not provide adequate specimen for diagnosis. Xishuang et al. found it more difficult to obtain intact tumor for precise TNM staging when doing Holmium *en bloc* resection (Hol-EBR) versus cTURBT. Smaller tumors, especially those 5 mm or less in diameter, may be most prone to vaporization effects [15]. Leaving a margin (anywhere from 2 to 20 mm around the tumor base) when resecting can minimize the risk of inadequate specimen [15, 21, 22]. He et al. was able to lessen this risk by using a front-firing rather than a side-firing green light laser [12]. Specimen can be further kept intact by using lower power settings. With green light laser, for instance, the standard 80–120 W power used for BPH procedures is not needed, and 30 W power is adequate [12, 15]. The concern for serosal injury is less with thulium lasers than with holmium and green light. Thulium lasers, also known as 2-micron continuous-wave laser systems, evaporate tissues continuously and do not generate pressure waves. As a result, the laser affects tissues only within 2 mm in front of the fiber tip, and cleaner resection cuts are achieved [22, 23]. Muto et al. found detrusor muscle to be present in all bladder tumor specimen resected with thulium laser in a prospective study on Thulium EBR [24]. An advantage of laser energy is that it does not activate the obturator nerve reflex as much as electroresection. Compared with cTURBT, studies usingr Hol-EBR and Thul-EBR have not reported cases of activating the obturator nerve reflex and thus mitigating the risk of bladder perforation [15, 21–23, 25].

Special Staging Circumstances: Diverticulae

Bladder diverticula present a unique staging challenge. They account for just 1% of all bladder cancers [26]. The majority of intra-diverticular bladder tumors (IDBTs) in adults are acquired, which means they lack true muscularis propria. Per the 2017 American Joint Committee on Cancer (AJCC) recommendations, stage T2 is omitted with IBDTs to avoid confusion since there is no muscle layer, [26, 27]. Normally, specimen quality is judged based on the presence of detrusor muscle, but this is lost with IDBTs. Previous studies have noted a dense fibroconnective band that demarcates the boundary between the lamina propria and perivesical fat [26, 28]. Invasion beyond this thick band has been suggested to be at least stage T3 [28]. Given that tumors can more easily extend from the lamina propria to the perivesical fat in a diverticulum, obtaining adequate specimen is especially important for staging [29]. This may be challenging with the increased risk of bladder perforation in a thin-walled diverticulum or difficulty passing an endoscope through a narrow diverticulum stalk.

Bimanual Exam

An exam under anesthesia can provide important information about the patient's clinical stage and tumor resectability and should be done before and after TURBT. Factors that suggest locally advanced disease include a fixed mass (cT4b),

invasion of adjacent structures (cT4a), and the presence of a large residual mass after TURBT (cT3b) [30]. While these are clinically useful benchmarks, results of the bimanual exam are not always accurate with one study reporting clinical overstaging in 11% and understaging in 33% of cases (total of 44% discordance) [31].

Accuracy of Staging: Importance of Repeat TURBT

As noted above, a single TURBT may provide inaccurate staging information. Most important is the risk for understaging, as this risks undertreatment of aggressive disease. Further, any intravesical therapy is most efficacious after complete tumor resection. A repeat resection is helpful in two ways: it eliminates any residual tumor at the operative site and allows for more accurate staging.

There is significant evidence supporting this practice. Herr and Donat from Memorial Sloan Kettering (MSK) showed high rates of residual tumor in patients referred after outside TURBT (74%), and there were significant rates of upstaging in both Ta (15%) and T1 (30%) tumors [32, 33]. Of note, this benefit did not extend to patients with low-grade disease at initial resection. These results were confirmed in a systematic review by Cumberbatch et al., which noted residual tumor in up to 70% of repeat resections and upstaging in up to 8% of Ta and 32% of T1 tumors [34]. This is true even for experienced clinicians. An MSK series of re-resection of T1 tumors in which initial resection was done at their center found persistent T1 tumor in 25% of patients [35].

Appropriate staging as above improves patient selection for appropriate therapies, whether it be intravesical adjunctive therapies or radical cystectomy. This improvement in patient selection translates into improved patient outcomes. A prospective trial by Divrik et al. showed that patients undergoing repeat TUR had improved recurrence-free and progression-free survival [36]. Further, repeat TURBT has demonstrated improved outcomes after BCG therapy. In both cases, it appears that repeat TURBT allowed for the full eradication of tumor and appropriate patient selection, which improved patient outcomes after therapy [37].

American Urological Association (AUA) Guidelines recommend repeat resection if there is incomplete resection at the initial TURBT or if there is T1 disease at initial resection. These same guidelines suggest consideration of repeat TURBT for high-risk, high-grade Ta tumors [30]. NCCN guidelines suggest similarly, adding a strong consideration for repeat TURBT in high-grade tumors if no detrusor muscle was present in the initial specimen [38]. This addition by the NCCN guidelines reflects the presence of detrusor muscle in the specimen as a proxy for adequacy/quality of initial TURBT. Indeed, several studies have shown decreased rates of understaging in patients with detrusor muscle in the primary specimen [10, 39]. The timing of the repeat TURBT has been debated in the literature, but most advocate for a time period of 4–6 weeks after initial TURBT. A retrospective study of high-risk NMIBC looking at the timing of re-resection showed decreased recurrence-free and progression-free survival in patients with repeat resection >42 after the initial resection [40].

Imaging

Imaging studies are an important component of the staging workup for both NMIBC and MIBC (see also Chap. 5). With any newly diagnosed bladder tumor, it is recommended to obtain cross-sectional imaging prior to planned OR for TURBT. This imaging should assess the upper urinary collecting system. Traditionally, this has been accomplished with triphasic CT scan, combining a non-contrast, arterial, and delayed (excretory) phase imaging. However, in some cases, such as allergy to CT contrast dye or compromised renal function, practitioners may choose to use triphasic MR imaging or retrograde urography at the time of cystoscopy in combination with non-contrast CT or ultrasound. Often, this has been completed previously if the patient's initial presentation was for gross or microscopic hematuria.

Obtaining this imaging is beneficial in several ways. If pathology is noted on this imaging, then the practitioner may choose to bypass the office cystoscopy and proceed directly to the operating room. Also, if upper tract findings are noted, they may be evaluated and addressed at the time of the TURBT. Further imaging is dependent on pathology noted after resection. In both NMIBC and MIBC, imaging of the upper tracts must be performed as above if not done previously. If MIBC is noted, addition of chest imaging (CT or X-ray) should be performed, and consideration for bone imaging should be given if the patient has concerning symptoms.

The use of MRI has become more common by practitioners given its useful soft tissue discrimination. Much work is underway to use MRI as a potential discriminator of muscle-invasive disease. Vesical Imaging-Reporting and Data System (VI-RADS) has been recently created to give a standardized reporting platform for clinicians [41]. While the study is still ongoing, there is hope MRI will provide a noninvasive method to assess muscle invasion or determine treatment response. Some initial data suggests the ability to determine between NMIBC and MIBC states, and further literature on the subject will provide more useful guidance as to the best use of this imaging [42]. Studies in the United Kingdom that are using mpMRI as a means of triaging patients with definitive muscle-invasive disease to immediate RC are underway (BladderPath Study).

The use of positron emission tomography (PET) may) be useful in the staging workup for bladder cancer (see also Chap. 7). Classically, the use of this modality has been limited, given that urinary excretion of tracer obscures assessment of the primary or upper tract tumors. However, some have advocated the use of this for assessment of lymph node metastases in MIBC. Dason et al. compared preoperative PET imaging to pathologic node metastases at the time of radical cystectomy and found PET imaging to be most useful in the setting of clinically enlarged nodes on cross-sectional imaging [43]. The most appropriate use of PET imaging as well as the most useful tracer is still undetermined.

Staging

Current TNM Staging

Staging for bladder cancers follows the tumor, node, metastasis (TNM) system (Table 4.4).

Table 4.4 Standard TNM staging for bladder cancer [44]

T – Primary tumor		
Tx	Primary tumor cannot be assessed	
T0	No evidence of primary tumor	
Ta	Noninvasive papillary carcinoma	
Tis	Carcinoma in situ: “flat tumor”	
T1	Tumor invades subepithelial connective tissue	
T2	Tumor invades the muscle	
	T2a	Tumor invades superficial muscle (inner half)
	T2b	Tumor invades deep muscle (outer half)
T3	Tumor invades perivesical tissue	
	T3a	Microscopically
	T3b	Macroscopically (extravesical mass)
T4	Tumor invades any of the following: prostate stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall	
	T4a	Tumor invades prostate stroma, seminal vesicles, uterus, or vagina
	T4b	Tumor invades pelvic wall or abdominal wall
N – Regional lymph nodes		
Nx	Regional lymph nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, presacral)	
N2	Metastasis in multiple regional lymph nodes in the true pelvis (hypogastric, obturator, external iliac, presacral)	
N3	Metastasis in a common iliac lymph node(s)	
M – Distant metastasis	No distant metastasis	
	M1a	Non-regional lymph nodes
	M1b	Other distant metastasis

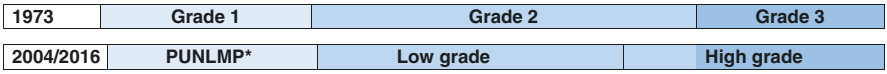

Fig. 4.1 WHO grading of NMIBC in 1973 versus 2004/2016 [47]. *PUNLMP papillary urothelial neoplasm of low malignant potential

Historical Considerations and Updates

In 1973, the World Health Organization (WHO) published its initial grading system for urothelial carcinomas based on cell differentiation. A more uniform system was adopted in 2004 (updated in 2016) that eliminated the heterogenous Grade 2 (G2) category. Figure 4.1 shows the shift in grading and result of the reclassification on each category. A systematic review did not find the 2004/2016 system outperformed the 1973 one in predicting recurrence and progression [45]. In fact, the 1973 groupings may more accurately predict recurrence and progression for pT1 tumors. In tumors classified as T1HG, Pelluchhi et al. found a higher recurrence (68% vs 50%) and progression rate (28% vs 9%) in the Grade 3 (G3) over G2 tumors, respectively [46].

Risk Stratification Schema

The creation of risk categories is helpful for counseling and surveillance. The AUA and EAU have separate but similar guidelines on this. The AUA stratifies risk into three categories (Table 4.5). Unique to the AUA risk table is the reclassification of intermediate-risk patients to high risk if they fail BCG treatment with the thought that these patients likely harbor more aggressive disease with increased risk for progression [30]. The EAU further subcategorizes high-risk patients into those with the highest risk [1]. This group includes tumors that are T1HG with concomitant bladder or prostatic urethra CIS, multiple and/or large T1HG and/or recurrent T1HG disease, variant histology, or lymphovascular invasion. Along with assigning a clinical stage, clinicians should designate each tumor recurrence or occurrence as low, intermediate, or high risk.

Current EAU NMIBC guidelines recommend using EORTC risk tables to determine a patients' risk to recur or progress after undergoing TUR. The tables are the result of a combined analysis of 2596 patients who underwent different prophylactic treatments after TUR of Ta-T1 bladder cancer with or without CIS. Each patient was assigned a total score (0–23) based on the presence or absence of certain factors influencing recurrence and progression. Higher scores indicate a worse prognosis (Table 4.6) [48]. Patients were then placed into one of four groups that related total score to 1- and 5-year recurrence and progression percentages (Table 4.7) [48]. Limitations of this study should be noted though. Specifically, no patients underwent second-look TUR or maintenance BCG, and only 78% received intravesical

Table 4.5 AUA Guidelines: Risk stratification for NMIBC [30] stratification for NMIBC

Low risk	Intermediate risk	High risk
LG[a] solitary Ta ≤ 3 cm	Recurrence within 1 year, LG Ta	T1 HG
PUNLMP[b]	Solitary LG Ta >3 cm	Any recurrent, HG Ta
	LG Ta, multifocal	HG Ta, >3 cm (or multifocal)
	HG[c] Ta, ≤3 cm	Any CIS[d]
	LG T1	Any BCG failure in HG patient
		Any variant histology
		Any LVI[e]
		Any HG prostatic urethral involvement

[a]*LG* low grade
[b]*PUNLMP* papillary urothelial neoplasm of low malignant potential
[c]*HG* high grade
[d]*CIS* carcinoma in situ
[e]*LVI* lymphovascular invasion

Table 4.6 EORTC series: weighted system to calculate disease recurrenceand progression scores

Factor	Recurrence	Progression
Number of tumors		
Single	0	0
2–7	3	3
≥8	6	3
Tumor size		
<3 cm	0	0
≥3 cm	3	3
Prior recurrence rate		
Primary	0	0
≤1 rec/yr	2	2
>1 rec/yr	4	2
T category		
Ta	0	0
T1	1	4
CIS		
No	0	0
Yes	1	6
Grade		
G1	0	0
G2	1	0
G3	2	5
Total score	0–17	0–23

Table 4.7 EORTC series: probability of disease recurrence and progression based on total score

Recurrence score	**Probability recurrence 1 year (95% CI)**	**Probability recurrence 5 years (95% CI)**
0	15% (10%, 19%)	31% (24%, 37%)
1–4	24% (21%, 26%)	46% (42%, 49%)
5–9	38% (35%, 41%)	62% (58%, 65%)
10–17	61% (55%, 67%)	78% (73%, 84%)
Progression score	**Probability progression 1 year (95% CI)**	**Probability progression 5 years (95% CI)**
0	0.2% (0%, 0%)	0.8% (0%, 1.7%)
2–6	1.0% (0.4%, 1.6%)	6% (5%, 8%)
7–13	5% (4%, 7%)	17% (14%, 20%)
14–23	17% (10%, 24%)	45% (35%, 55%)

therapy [48]. Nowadays, many patients with high-risk disease undergo re-resection and maintenance BCG, which both independently reduce recurrence and progression [40, 49, 50].

The Spanish urologic oncology group (CUETO) subsequently performed external validation of the EORTC model. In their study, all patients had NMIBC treated with 12 intravesical instillations of BCG over 5–6 months [51]. They found that EORTC tables overestimated the risk of progression in high-risk patients and risk of recurrence overall after BCG. In the EORTC study, high-risk patients had a 61% and 78% chance to recur at 1 and 5 years compared with 26–30% and ~50% in the CUETO group, respectively [48, 51]. Chance of progression in EORTC high-risk patients was 17% and 45% at 1 and 5 years versus the 14% and 34% seen in the comparative CUETO patients [48, 51]. These reduced risks are likely due to treatment with BCG which has been shown to lessen recurrences and progression to MIBC [49, 50]. Notably, study populations were slightly different, with more G3 T1 tumors and CIS seen in the CUETO series.

Surveillance Protocols

Non-muscle-Invasive Disease

The goal of surveillance in NMIBC is to detect high-grade recurrence and progression to MIBC as early as possible after the initial treatment in order to optimize patient outcomes. Low-grade tumors rarely progress, so early detection is less essential. In contrast, intermediate-/high-grade tumors progress more frequently, so timely detection is key as diagnostic delays can be life-threatening. Overall, there is a lack of robust randomized trial data comparing different surveillance strategies to suggest one over the other. The EORTC and CUETO risk stratification models provide a starting point that can be adapted based on each patient's individual risk.

Cystoscopy remains the standard for surveillance follow-up. As with diagnosis, urine cytology should be used as an adjunct. Currently, no urinary biomarkers, cytology, or imaging studies can replace the ability to carefully inspect the patient's entire bladder. Visualization allows the clinician to ensure the previous resection was complete and look for new tumor. The first cystoscopy after TURBT (and any adjuvant therapies) is generally recommended to be done at 3–4 months. Multiple studies have found recurrence at 3 months to be an important predictor of future recurrences and progression to MIBC [52, 53]. This is especially important for TaT1 tumors or CIS, which are independent risk factors for progression [52, 53]. The AUA defines three surveillance strategies after a negative first cystoscopy [30]:

- Low risk – Next cystoscopy at 6–9 months and then annually
- Intermediate risk – Next cystoscopy and cytology at 3–6 months for 2 years, 6–12 months for years 3 and 4, and then annually
- High risk – Next cystoscopy and cytology every 3–4 months for 2 years, 6 months for years 3 and 4, and then annually

The less frequent surveillance for low-risk disease is supported by a historical study by Olsen & Genster et al., the only RCT on follow-up in NMIBC. Though the study was small, it showed no difference in recurrence, progression, or survival between more or less frequent follow-up for low-risk NMIBC (every 3 months versus 6 months) [54]. In a retrospective study, Shroeck et al. similarly found that among patients with Ta disease, low-intensity surveillance (≤5 cystoscopies over 2 years) was not associated with increased risk of disease progression to T1/T2 or death from bladder cancer when compared with high-intensity surveillance [55]. Furthermore, frequent cystoscopies (>3 in 2 years) among the low-risk NMIBC group are associated with twice as many TURs without a decrease disease progression or death [56]. Both AUA and EAU guidelines suggest discontinuing surveillance of low-risk patients after 5 years if disease-free due to the limited recurrences and muscle progression noted past this point [1, 30]. In a study by Matsumoto et al., 14.9% of patients who had undergone TURBT for NMIBC had any recurrence after a disease-free interval of 5 years, and none were in patients with low-grade Ta [57]. Recurrence past 5 years is not uncommon but those at low risk likely do not require lifelong follow-up. Further, active surveillance with urine cytology appears to be a safe approach for small, recurrent, low-grade Ta tumors [58]. This may be especially beneficial in elderly patients.

The duration of follow-up is less clear for patients with intermediate- or high-risk disease. The EAU recommends life-long follow-up, while the AUA recommends shared decision-making after 5 years of being disease-free [1, 30]. Both groups agree intermediate- and high-risk patients should have upper tract imaging periodically (every 1 or 2 years) [1, 30]. In an observational study of 1529 patients with NMIBC, Millán-Rodríguez et al. found an increasing incidence of UUT with increasing risk group (low risk: 0.6%, intermediate: 1.8%, high: 4.1%) [59]. Aside from higher-risk groups, having multiple recurrent superficial bladder tumors has

been found to be another predictor for the development of upper urinary tract tumors (UUT) [59].

Recurrence After BCG

Despite several additional intravesical therapies available on the market, BCG remains the standard of care for patients with high-grade NMIBC and CIS. While BCG has been shown to reduce both recurrence and progression, a number of patients will experience treatment failure. In order to characterize the diverse situations in which this can occur, the International Bladder Cancer Group (IBCG) has a standardized classification of BCG failure into four different categories: refractory, relapsing, intolerant, and unresponsive [60].

- *Refractory*: persistent high-grade disease at 6 months despite adequate BCG treatment
- *Relapsing*: recurrent high-grade disease after previously achieving disease-free state at 6 months after adequate BCG (or last exposure)
- *Intolerant*: disease persists due to inability to receive adequate BCG because of toxicity
- *Unresponsive*: includes patients with BCG refractory or relapsing (within 6 months of last BCG) disease

There are a few points to emphasize regarding the above classification. First, the impetus for waiting 6 months prior to classification as BCG-refractory stems from the knowledge that a significant proportion of patients will respond to a second course of BCG [61]. Second, the designation of BCG-unresponsive disease (BCG refractory or relapsing within 6 months) should denote to the practitioner that further BCG is unlikely to be efficacious.

Conclusion

The spectrum of potential treatments for newly diagnosed bladder cancer is quite broad, spanning from close observation for low-grade lesions to radical surgery in the context of muscle-invasive disease. Selection of the appropriate treatment first involves obtaining accurate staging. Through the use of quality bladder resection, physical examination, and imaging, the practitioner can accurately determine the appropriate stage, weigh prognostic risk, and evaluate available treatment options.

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Chapter 5
Conventional and Investigational Imaging Modalities

Ersan Altun

Introduction

Cystoscopy with transurethral bladder tumor resection is the primary diagnostic direct visualization technique for the initial diagnosis of bladder cancer [1, 2]. Since the most common presentation of bladder cancer is hematuria, cystoscopy is recommended by the American Urological Association for bladder evaluation, and therefore, the role of diagnostic imaging is limited in the initial diagnosis [1, 2]. However, imaging of bladder cancer is critical for local and distant staging, detection for metachronous and synchronous upper tract urothelial carcinoma, assessment of treatment response, evaluation of disease, and treatment-related complications [1–4].

Additionally, recently proposed multiparametric MRI staging of bladder cancer may also be used to identify muscle-invasive disease, although the specific clinical role of this technique has not been established yet [5]. Furthermore, newly developing radiomics techniques may also have a role in the imaging evaluation of bladder cancer [6].

Role of Imaging in the Diagnosis of Bladder Cancer and Directing the Therapeutic Approach

The initial diagnosis of bladder cancer is usually made during hematuria workup. According to the American Urological Association, in patients with gross or visible hematuria and high-risk microscopic hematuria, in addition to cystoscopy,

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assessment of the upper tract is performed by cross-sectional urographic imaging techniques including CT and MR urography [1]. The use of renal ultrasound is recommended in patients with intermediate-risk microscopic hematuria in addition to cystoscopy [1]. Repeat urine analysis in 6 months versus cystoscopy and renal ultrasound is recommended for the patients with low-risk microscopic hematuria [1].

Upper tract imaging with CT and MR urography is performed since synchronous urothelial carcinoma is seen in 2% of the patients with bladder urothelial carcinoma, and metachronous urothelial carcinoma is seen in 3.9% of the patients with history of bladder cancer [3]. CT urography is the most commonly used technique for imaging of upper tract [1, 2, 7]. However, if there is history of severe allergy or anaphylaxis to iodinated contrast media, MR urography could be performed with gadolinium-based contrast media [8]. If there is history of moderate allergic reactions to IV contrast agents, preprocedural preparation of patients is recommended with the use of steroid regimens [9]. If the patient is on dialysis without concern for any residual renal function, CT including precontrast and postcontrast imaging could be performed although the assessment could be limited due to the lack of excretory phase secondary to poor renal function [10]. MRI with contrast should not be preferred in patients undergoing dialysis or acute renal failure, and stable agents should cautiously be used in patients with stage 4 and 5 chronic kidney disease [11]. Alternative imaging techniques including renal ultrasound or noncontrast MRI also employing noncontrast urography techniques could be performed if CT or MR could not be performed due to pregnancy, history of severe allergic reactions, anaphylaxis, renal impairment, or the presence of incompatible implants with MR imaging or implants impairing image quality on CT and MRI [1, 2, 8, 10, 11]. The details of alternative imaging approaches are summarized in Table 5.1. A retrograde pyelogram could also be performed if the findings on imaging are inconclusive [1, 2].

Besides the detection of upper tract disease, cross-sectional imaging with CT and MRI is also used for local and distant staging [12–14]. Histopathologic staging is performed with the TNM system published as the 8th edition of the American Joint Committee on Cancer in 2018 [15]. Urothelial carcinoma is the most common type of bladder cancer forming 90% of the bladder cancers in the Western world [2, 3, 13, 14]. Squamous cell carcinomas and adenocarcinomas forming 6–8% and 2% of the bladder cancers are rare in the Western world [2, 3, 13, 14]. Squamous cell bladder cancer is the major type seen in developing countries where schistosomiasis is endemic [2, 14]. Squamous cell carcinomas and adenocarcinomas are aggressive tumors and usually present with advanced disease [14].

Cross-sectional imaging with CT and MRI is is used to assess the treatment response including tumor burden, lymph node involvement, and distant metastases following TURBT, chemotherapy, immunotherapy, and radiotherapy and surgery.

Surveillance with cross-sectional imaging following treatment of non-muscle-invasive bladder cancer is performed at every 1–2 years for the assessment of upper tract in patients with intermediate (including recurrence of low-grade Ta within 1 year, solitary low-grade Ta >3 cm, high-grade Ta ≤3 cm, and low-grade T1) and high (high-grade T1, recurrent high-grade Ta, high-grade Ta >3 cm or multifocal high-grade disease, carcinoma in situ, BCG failure in high-grade disease,

Table 5.1 Alternative imaging approaches when contraindications and relative contraindications to specific imaging modalities are present

	CT urogram	MR urogram	Noncontrast MRI or renal ultrasound
Severe allergic reaction or anaphylaxis	MR urogram could be done if there is history of severe allergy or anaphylaxis to iodinated contrast	CT urogram could be done if there is history of severe allergy or anaphylaxis to gadolinium-based contrast	Noncontrast MRI or CT or renal ultrasound could be performed if there is history severe allergy to both types of contrast
Renal impairment and dialysis [10, 11]	IV contrast use is not recommended in patients with acute renal failure The risk of CIN significantly increases in patients having eGFR[a] less than 30 ml/min/1.73 m^2. IV contrast should be cautiously used in this patient group if its use is essential and periprocedural IV hydration could be helpful to decrease the risk of contrast-induced nephropathy Iodinated contrast media can be used if there is no residual renal function but there will be no urographic component	IV contrast use is not recommended in patients with acute renal failure, patients with end-stage renal disease, or patients undergoing dialysis due to risk of nephrogenic systemic fibrosis and gadolinium deposition concerns. IV contrast should be cautiously used in patients with stage 4–5 chronic kidney disease not undergoing chronic dialysis Stable gadolinium agents are used in patients having eGFR less than 30 ml/min/1.73 m^2	Nonconrast MRI or CT or renal ultrasound can be used if clinical questions could be answered without the use of IV contrast
Incompatible implants or implants causing significant artifacts and image degradation	Depending on the type of implant, MRI, or MR urogram could be considered	Depending on the type of implant, CT urogram could be considered	Renal ultrasound could also be considered if the artifacts are present on both MRI and CT
Pregnancy	Nonconrast MRI or renal ultrasound should be performed	Nonconrast MRI or renal ultrasound should be performed	N/A

[a]Estimated glomerular filtration rate

lymphovascular invasion, high-grade prostatic urethral involvement) risk non-muscle-invasive disease [2]. For low-risk non-muscle-invasive disease, no routine upper tract imaging is recommended [2]. Surveillance with cross-sectional imaging for nonmetastatic muscle-invasive bladder cancer following cystectomy is performed with CT or MR urography and chest radiography or chest CT every

3–6 months for 1–2 years, CT/MR and chest radiography or chest CT annually for 3–5 years, and renal ultrasound for 5–10 years [2]. Surveillance with cross-sectional imaging for nonmetastatic muscle-invasive bladder cancer following bladder sparing treatment is performed with CT or MR urography and chest radiography or chest CT every 3–6 months for 1–2 years and CT/MR and chest radiography or chest CT annually for 3–5 years [2]. PET/CT with FDG could be performed for further assessment if metastatic disease is suspected during the surveillance [2]. There are no specific guidelines for the surveillance of metastatic muscle-invasive bladder cancer, although short-term follow-up with 3–6 month intervals is also preferred [2]. Contrast-enhanced studies are done for the abdomen and pelvis if there is no contraindication to IV contrast. If chest CT studies are done without concurrent CT studies for the abdomen and pelvis, IV contrast use is not necessary for the assessment of the chest.

Cross-sectional imaging with or without urography is also essential for the assessment of disease or treatment-related complications including but not limited to bladder rupture following TURBT, fistulization to the adjacent organs such as vagina or rectum, anastomotic leaks following urinary diversion or partial cystectomy, anastomotic strictures, and reflux from the conduit into the ureters and kidneys.

Imaging Modalities

Ultrasound

Imaging Technique

Bladder ultrasound is performed by using a 2–5 megahertz (MHz) convex probe [3]. In order to be able to assess the bladder wall and lumen, the bladder has to be distended at least moderately with urine or fluid. The bladder volume of 250–500 ml is usually sufficient for optimal bladder distention. If the bladder is not distended, US assessment will be extremely limited or nondiagnostic. About 500–1000 ml of fluid intake is encouraged 1 hour before the bladder US. In patients with indwelling urinary catheter, 250–500 ml saline is administered to the bladder through the catheter to provide bladder distension if the patient is able to tolerate. Color Doppler US technique is also used to detect vascularity in suspicious bladder lesions [3]. Reverberation artifacts, side lobe artifacts, section thickness artifacts, and range ambiguity artifacts may impair the image quality and adversely affect the detection of focal lesions during bladder US [16]. Reverberation artifacts and side lobe artifacts could be avoided by using tissue harmonic imaging, changing the angle of insonation, and decreasing gain [16]. Section thickness artifacts can be avoided by placing the area of interest in the focal zone [16]. Range ambiguity artifacts can be avoided by reducing the number of focal zones and increasing the image depth [16].

Imaging Role, Clinical Impact, and Accuracy of US

Bladder cancer can be incidentally detected during evaluation for other reasons including but not limited to lower urinary tract symptoms, infection, and urinary retention. Bladder ultrasound is also done to assess the presence and size of potential intraluminal bladder hematoma secondary to hematuria.

Bladder cancer can present as diffuse bladder wall thickening, focal bladder wall thickening, polypoid bladder mass, or sessile focal lesions along the bladder wall [3]. The lesions may demonstrate variable echogenicity and usually irregular contours [3]. Calcifications could be seen in the lesions. Hematomas could potentially be differentiated from bladder cancer with the help of mobility during the real-time ultrasound examination. Hematomas usually demonstrate mobility with the movement of patients, while bladder cancer lesions are immobile. Vascularization could be detected in bladder cancer lesions with color Doppler. Muscle-invasive cancer can be diagnosed when the muscular hypoechoic middle layer of the bladder is involved with tumor. The normal inner mucosal and outer serosal layers of the bladder are seen as hyperechoic layers.

The detection and assessment of muscle involvement with staging of bladder cancer are limited due to low soft tissue contrast resolution, inadequate bladder distension, and operator dependence [3]. The sensitivity and specificity for the detection of bladder cancer with ultrasound are variable and have been reported to vary between 60.9% and 63% and 72.1% and 99%, respectively [3].

Computed Tomography

Imaging Technique

CT is the most commonly used technique for the assessment of bladder cancer including detection and staging [14]. CT urography is the preferred method for the evaluation of bladder cancer during the initial diagnosis and surveillance at least for 1–2 years due to the ability to assess locoregional disease, lymph nodes, distant metastasis, and potential upper tract disease [1, 2, 13, 14]. CT urography includes the noncontrast phase of the abdomen and nephrographic phase and excretory phase of the abdomen and pelvis (Fig. 5.1) [4, 7, 13, 17, 18]. The noncontrast phase is usually acquired for the abdomen only to decrease radiation exposure. However, virtual noncontrast series of the abdomen and pelvis could also be created without additional exposure to radiation if dual-energy CT techniques are used [7, 17]. The nephrographic phase is usually acquired at 70 seconds after contrast administration, and the excretory phase is usually acquired at 5–6 minutes after contrast administration [17]. Split bolus technique which is used at some institutions performs CT urography with the administration of contrast bolus at two different times. By split bolus technique, in addition to noncontrast phase, nephrographic and excretory phase images are acquired at the same time, which overall decreases radiation

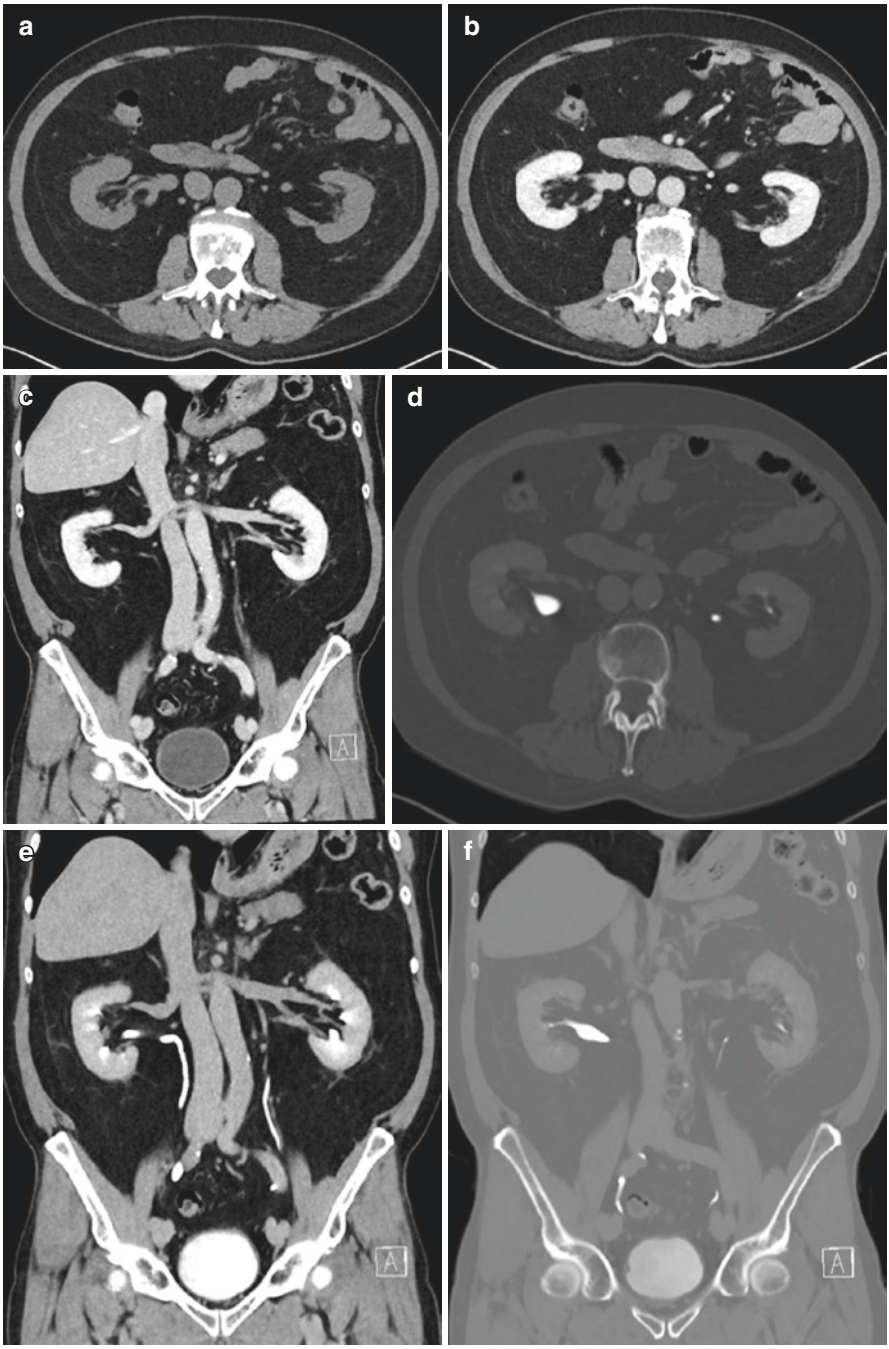

Fig. 5.1 Normal CT urogram. Transverse noncontrast (**a**), transverse nephrographic phase (**b**), coronal nephrographic phase (**c**), transverse excretory phase (**d**), and coronal excretory phase (**e**, **f**) CT images showing a sample triphasic CT urogram acquired at 64-slice single energy CT scanner. Please note that intermediate window setting is preferred for the assessment of contrast-filled renal collecting system, ureters and bladder for the detection of focal lesions

exposure and scanning time [7, 18]. However, it has been reported that the inability to assess the enhancement of the walls of the renal collecting system, renal pelvis, ureters, and bladder wall due to masking effect of excreted contrast limit the detection of small lesions [13]. Sample CT urography protocols are given in Table 5.2.

Imaging Role, Clinical Impact, and Accuracy of CT

In diagnostic workup of suspected bladder cancer, CT urography is performed in combination with cystoscopy and transurethral bladder tumor resection (if there is any lesion) for the detection and staging of bladder cancer (Fig. 5.2) [1, 2]. Sensitivity, specificity, and accuracy of CT urography have been reported to be 79%, 94%, and 91% for the detection of bladder cancer [4, 13]. CT urography is

Table 5.2 CT urography technique

	Triple phase CT urography with single energy CT (64 slice)	Dual phase split bolus CT urography with single energy CT (64 slice)	Dual phase CT urography with dual energy CT (2 × 128 slice)
kVp/effective mAs/rotation time (sec)	120/250/0.33	120/250/0.33	120/290/0.5
Detector collimation (mm)	0.6	0.6	0.6
Slice thickness (mm)	3	3	3
Pitch	0.75	0.75	0.6
Image acquisition	Craniocaudal	Craniocaudal	Craniocaudal
Contrast volume	1.5–1.7 ml/kg (max 100–120 ml)	1.5–1.7 ml/kg (max 100–120 ml)	1.5–1.7 ml/kg (100–120 ml)
Contrast dose	300–350 mg iodine/ml of the contrast	300–350 mg iodine/ml of the contrast	300–350 mg iodine/ml of the contrast
Injection rate of IV contrast	3–4 ml/sec	3–4 ml/sec	4–5 ml/sec
Scan phases and delays	Noncontrast phase. Nephrograhic phase. (60–70 seconds after the IV contrast administration) Excretory phase. (6–8 minutes after the IV contrast administration)	Noncontrast phase. Combined Nephrographic and excretory phase (injection of 2/3 volume of the contrast followed by injection of 1/3 of contrast in 6–10 minutes. Acquisition is obtained at 100 seconds after the injection of second bolus)	Nephrographic phase. (60 seconds after the IV contrast administration) Excretory phase. (6–8 minutes after the IV contrast administration) Virtual nonenhanced images are also reconstructed
Multiplanar reconstructions	Axial, coronal, and sagittal	Axial, coronal, and sagittal	Axial, coronal, and sagittal
3D technique	MIP	MIP	MIP

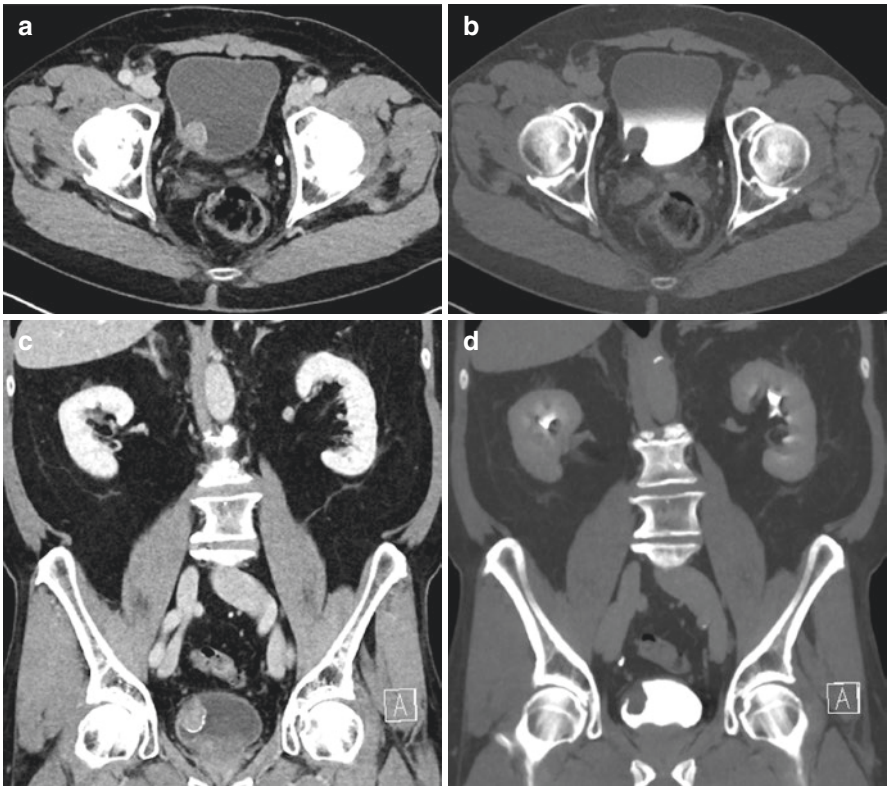

Fig. 5.2 Bladder cancer. Transverse (**a**) and coronal (**b**) CT images acquired at the nephrographic phase, and transverse (**c**) and coronal (**d**) CT images acquired at the excretory phase demonstrate a polypoid heterogeneously enhancing mass arising from the right posterolateral wall of the bladder and extending into the bladder lumen at the level of right ureterovesical junction. The mass is not obstructing since there is no obvious evidence of hydroureter or hydronephrosis. Please note the presence of calcifications at the periphery of the mass

particularly limited in the detection of small particularly flat and sessile lesions [14]. CT has overall low accuracy in T staging of bladder cancer, and it is especially limited to differentiate non-muscle-invasive and muscle-invasive disease if there is no obvious extravesical invasion. The sensitivity, specificity, and accuracy for the identification of T3 disease, i.e., bladder cancer with extravesical extension, vary between 62% and 89%, 63% and 100%, and 49% and 93% [3, 4, 13, 14].

CT is also limited in the staging of lymph nodes since CT assessment relies only on the size and morphology of the lymph nodes. Lymph nodes with a short-axis size larger than 1 cm are regarded as abnormal lymph nodes, and those with 0.8 cm are usually regarded as suspicious lymph nodes in the pelvis and retroperitoneum. Additionally, round morphology is usually regarded as abnormal particularly if the

lymph nodes are 0.8 cm and larger. Variable sensitivity, specificity, and accuracy have been reported for the detection of lymph node metastasis by CT ranging from 9% to 83%, 56% to 100%, and 54% to 86%, respectively [13].

CT urography is used for the identification of synchronous and metachronous upper tract urothelial carcinoma (Fig. 5.3) during the initial diagnostic workup and surveillance with sensitivity, specificity, and accuracy ranging between 93.5% and 95.8%, 94.8% and 100%, and 94.2% and 99.6% [19]. The positive predictive value of CT urography has also been reported to be 53% overall, 83% for large masses, 0% for small masses, and 46% for urothelial thickening [20].

CT urography is also able to assess distant metastatic disease including retroperitoneal lymph node and distant organ metastases in the abdomen and pelvis.

Magnetic Resonance Imaging

Imaging Technique

MRI of the bladder can be performed at 1.5 T or 3.0 T with phased-array body coils. No patient preparation is necessary for the examination unless the patient needs premedication due to prior history of moderate allergic reaction to gadolinium-based contrast media.

MR Urography

MR urography could be performed for the assessment of not only hematuria but also lower urinary tract symptoms, urinary obstruction (associated with bladder cancer), and synchronous and metachronous urothelial cancers [8, 12, 18, 21]. Additionally, MR urography can be used for surgical planning if there is additional complex history of prior surgeries or congenital anomalies [12]. IV administration of 250 ml of saline 15–30 minutes before the examination would be helpful to hydrate the patient [8, 12]. The patients are instructed to void just before the examination. MR urography sequences are performed following the IV administration of diuretic agent furosemide with the dose of 0.1 mg/kg or 5–10 mg for the adults if there is no contraindication to the diuretics [12]. The acquisition of MR urography sequences is performed on the coronal plane extending from the top of the kidneys to the level of the inferior border of the external urethral sphincter in the males and to the level of urethral meatus in the females. In addition to MR urography sequences, precontrast sequences including transverse and coronal T2-weighted single-shot echo train spin echo (SS-ETSE), transverse T2-weighted fat-suppressed SS-ETSE, transverse T1-weighted in-phase and out-of-phase gradient echo, transverse and coronal T1-weighted precontrast fat-suppressed gradient echo, and transverse diffusion weighted imaging sequences are acquired.

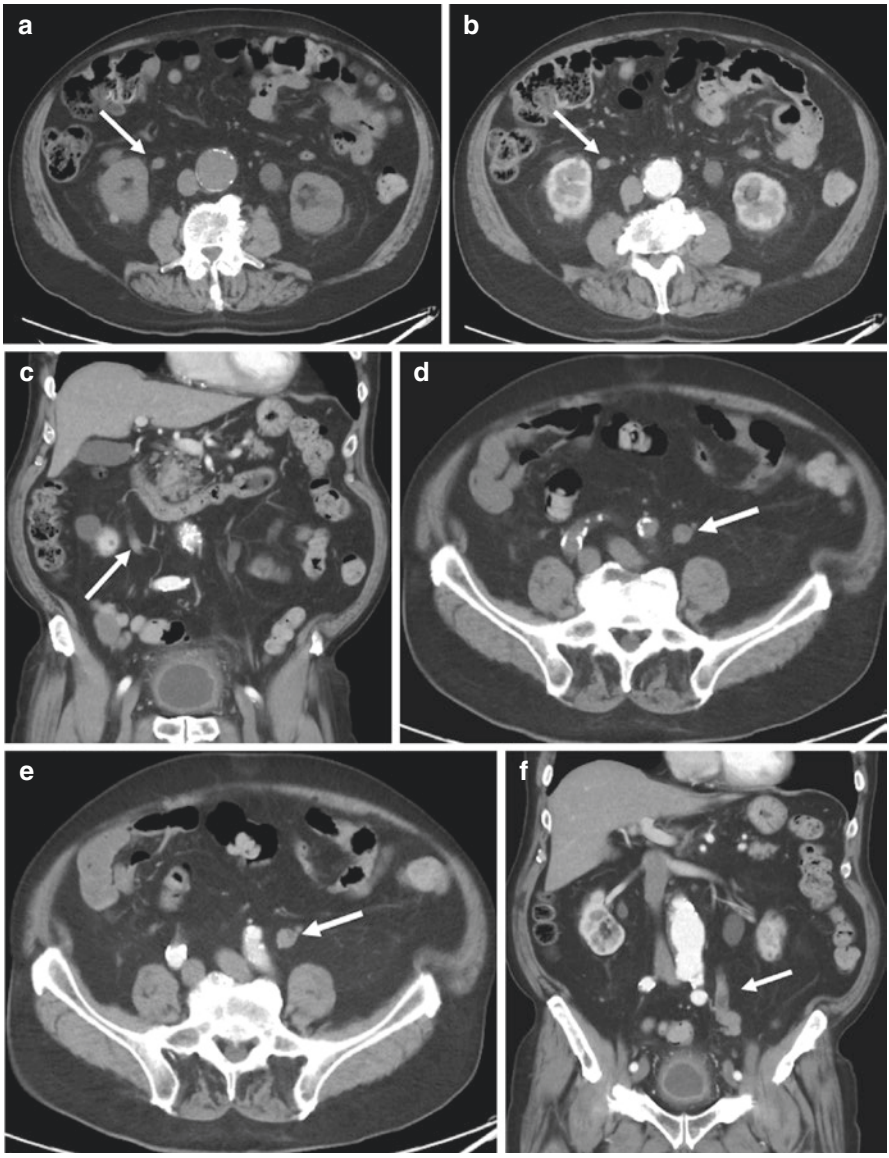

Fig. 5.3 Bilateral ureter masses. Transverse noncontrast (**a**), transverse (**b**), and coronal corticomedullary phase CT images show a right small enhancing ureter mass (arrows, **a**–**c**). Transverse noncontrast (**d**), transverse (**e**), and coronal (**f**) corticomedullary phase CT images show a left-sided larger enhancing ureter mass (arrows, **d**–**f**). The lesions are obstructive and associated with bilateral hydroureteronephrosis (not shown)

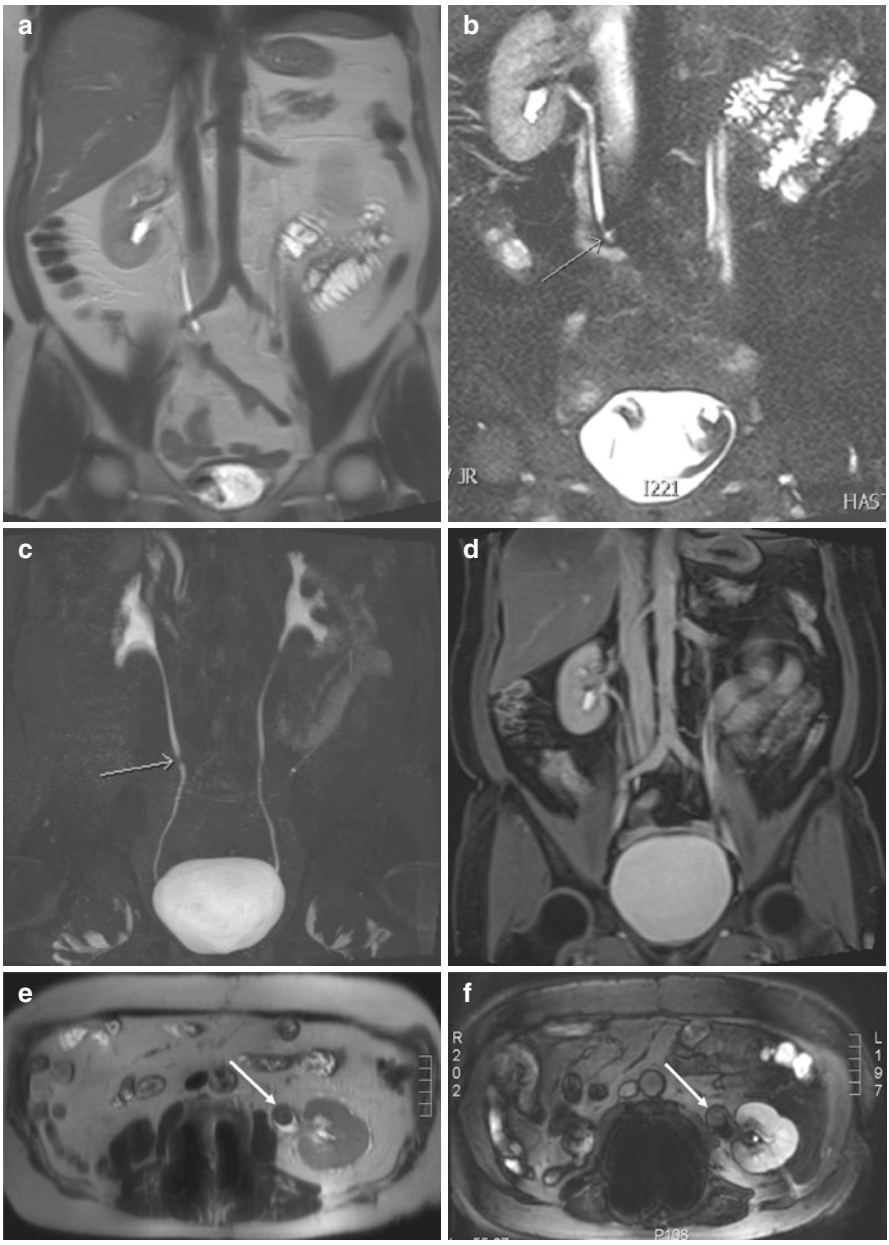

Fig. 5.4 MR urography. Coronal T2-weighted single shot echo train spin echo (SS-ETSE) (**a**), coronal T2-weighted thin slice fat-suppressed SS-ETSE (**b**), coronal T2-weighted thick-slab single shot turbo spin echo (**c**), coronal T1-weighted postgadolinium excretory phase three-dimensional gradient echo (3D GE) images demonstrate the compression of right ureter mildly due to right common iliac artery (thin arrows) on noncontrast fluid-based MR urography images (**b**, **c**) and postcontrast excretory phase MR urography (**d**) image. Ureteropelvic junction mass. Transverse T2-weighted SS-ETSE (**e**) and transverse T1-weighted fat-suppressed 3D GE (**f**) images demonstrate a left ureteropelvic junction mass (arrows, **e**, **f**)

Table 5.3 MR urography technique at 1.5 T

Sequence	Plane	Location	TR[a]	TE[b]	Flip Angle	Thickness/gap	FOV[c]	Matrix
Before diuretic administration – Precontrast sequences								
Localizer	3-plane							
SS-ETSE[d]	Coronal	Abdomen and pelvis	1500[e]	85	170	6 mm/20%	350–400	192 × 256
SS-ETSE	Axial	Abdomen and pelvis	1500[e]	85	170	6 mm/20%	350–400	192 × 256
SS-ETSE	Sagittal	Abdomen and pelvis	1500[e]	85	170	6 mm/20%	350	192 × 256
SS-ETSE fat-suppressed	Axial	Abdomen and pelvis	1500[e]	85	170	8–10 mm/20%	350–400	192 × 256
T1 SGE[f] in/out-of-phase	Axial	Abdomen and pelvis	170	2.2/4.4	70	7 mm/20%	350–400	192 × 320
T2 3D TSE	Axial	Pelvis	1200	120	150	1.5 mm	250	256 × 256
Diffusion weighted imaging	Axial	Abdomen and pelvis	4500	88	90	3 mm	270	128 × 128
T1 3D GE[g] fat-suppressed	Axial and coronal	Abdomen and pelvis	3.8	1.7	10	3 mm	350–400	160 × 256
After diuretic administration – Precontrast sequences								
T2-weighted MR urography	Coronal	Ureters and bladder	5000	700	180	50 mm	400	224 × 384
After diuretic administration – Postgadolinium sequences								

T1 3D GE fat-suppressed[h]	Axial/coronal/ sagittal	Abdomen and pelvis	3.8	1.7	10	3 mm	350–400	160 × 256

[a]TR: Repetition time
[b]TE: Echo time
[c]FOV: Field of view
[d]SS-ETSE: Single shot echo train spin echo
[e]TR between slice acquisitions
[f]SGE: Spoiled gradient echo
[g]3D GE: Three-dimensional gradient echo
[h]Coronal images covering the kidneys, ureters, and bladder should be acquired at 35, 70, 90, 120, 360–480 seconds after the contrast administration. Additionally, transverse and sagittal fat-suppressed three-dimensional gradient echo sequences could also be obtained on the nephrographic and excretory phases. IV contrast is administered at 2 ml/sec with the help of power-injector

MR urography studies can be performed by using two urographic techniques including (i) fluid-based T2-weighted imaging and (ii) contrast media-based excretory phase imaging employing T1-weighted imaging (Fig. 5.4). Sample MR urography protocol at 1.5 T is given in Table 5.3.

Fluid-based MR urography can be performed by using two-dimensional T2-weighted SS-ETSE sequences with thinner slices for the assessment of renal collecting systems particularly and single-shot thick slab heavily T2-weighted turbo spin echo (TSE) for the assessment of ureters particularly. The fluid-based MR urography technique is especially critical in pregnant patients since ionizing radiation and gadolinium-based contrast agents cannot be used.

Contrast-based MR urography is performed using three-dimensional gradient echo T1-weighted sequences following the IV administration of gadolinium-based contrast agents. Dynamic imaging is performed on the corticomedullary phase (35 seconds after the contrast administration), nephrographic phase (60–70 seconds after the contrast administration), and excretory phase (5–8 minutes after the contrast administration). Subtraction of precontrast series from postcontrast series could be particularly helpful for the creation of three-dimensional images. Additionally, transverse and sagittal fat-suppressed three-dimensional gradient echo sequences could also be obtained on the nephrographic and excretory phases.

Bladder Cancer Staging

MRI can also be used specifically for staging bladder cancer to differentiate muscle-invasive disease from non-muscle-invasive disease. The patient is instructed to void 60 minutes before the examination and given 500–1000 ml of fluid to drink 30–60 minutes before the examination to distend the bladder.

The key sequences for the staging of bladder cancer include high-resolution T2-weighted TSE sequence in three planes, diffusion weighted imaging (DWI)) and dynamic contrast-enhanced (DCE) postgadolinium three-dimensional gradient echo imaging of the bladder. These examinations should be performed with a small field of view, thinner slices (3–4 mm) without any intersection gap, and a high image matrix. DCE imaging is performed at the arterial phase, venous phase, interstitial phase, and excretory phase. Multiple repeated acquisitions through the bladder could be very helpful to assess the enhancement patterns of focal lesions during the arterial (25 seconds after the injection), venous (60 seconds after the injection), interstitial (120 seconds after the injection), and excretory phases (360 seconds after the injection).

In addition to these staging sequences, precontrast and postcontrast sequences for the assessment of the pelvis are also acquired. This is particularly important for the assessment of lymph node involvement, complications, and any additional chronic and incidental pathology or findings. A sample MRI protocol for bladder cancer staging is given in Table 5.4.

Table 5.4 MR technique for bladder cancer staging of pelvis at 1.5 T

Sequence	Plane	TR[a]	TE[b]	Flip angle	Thickness/gap	FOV[c]	Matrix
Localizer	3-plane						
SS-ETSE[d]	Coronal	1500[e]	85	170	6 mm/20%	350–400	192 × 256
SS-ETSE	Axial	1500[e]	85	170	6 mm/20%	350–400	192 × 256
SS-ETSE	Sagittal	1500[e]	85	170	6 mm/20%	350	192 × 256
SS-ETSE fat-suppressed	Axial	1500[e]	85	170	8–10 mm/20%	350–400	192 × 256
T1 SGE[f] in/ out-of-phase	Axial	170	2.2/4.4	70	7 mm/20%	350–400	192 × 320
T2 3D TSE	Axial	1200	120	150	1.5 mm	250	256 × 256
T2 TSE	Axial/ coronal/ sagittal	5000	80	90	3 mm	230	256x256
Diffusion weighted imaging	Axial	4500	88	90	3 mm	270	128 x 128
T1 3D GE[g] FS pre	Axial	3.8	1.7	10	3 mm	350–400	160 × 256
Post-gadolinium sequences							
T1 3D GE fat-suppressed[h]	Axial/ coronal/ sagittal	3.8	1.7	10	3 mm	250/350–400	160 × 256

[a]TR: Repetition time
[b]TE: Echo time
[c]FOV: Field of view
[d]SS-ETSE: Single shot echo train spin echo
[e]TR between slice acquisitions
[f]SGE: Spoiled gradient echo
[g]3D GE: Three-dimensional gradient echo
[h]Axial imaging should be first performed at 25 seconds after the contrast administration with a small FOV for the bladder. This sequence should be repeated 4–6 times every 25 seconds for the bladder in the axial and coronal planes consecutively after the first acquisition. Larger FOV images covering the pelvis should be acquired in 3 planes following the acquisition of small FOV images. IV contrast is administered at 2 ml/sec with the help of power-injector

Imaging Role, Clinical Impact, and Accuracy of MR

MR Urography

MR urography is an accurate imaging technique for the detection of bladder cancer during the workup of hematuria or lower urinary tract symptoms, and the sensitivity of MR urography (91%) is similar to that of CT urography (94%) [21]. Although MR urography has been reported to be moderately sensitive for the detection of upper tract disease (Fig. 5.4), the literature is still scarce, and MR urography has

been reported to have high sensitivity for the detection of upper tract disease and likely comparable to CT urography [18].

MR urography can be used in combination with MR angiography and standard MR imaging to assess the arterial supply of the kidneys, collecting system of the kidneys, ureters, renal parenchyma, and bladder in one examination for preoperative assessment. Additionally, MR urography can be used to assess postoperative complications following cystectomy or treatment of upper tract disease such as urinomas or urine leaks, fistulas involving the GU tract.

MR urography with noncontrast techniques can also be used in pregnant patients and in patients who are not able to get IV contrast due to renal impairment or allergic reactions.

Bladder Cancer Staging

Bladder cancer staging is performed by using T2-weighted high-resolution TSE sequences, DWI sequence and DCE sequence. The remaining sequences are used for the evaluation of lymph node involvement, possible distant metastases, and additional incidental findings.

T Staging

The muscularis propria is the predominant layer at the bladder wall and is hypointense on T2-weighted TSE, mildly hyperintense on high-value is the DWI showing intermediate signal intensity on ADC map, and hypointense on T1-weighted images. The mucosa and submucosa could not be differentiated on T2-weighted TSE and DWI images [5, 14, 22–27]. Early enhancement of the mucosa and submucosa on the arterial phase DCE images is visualized, while muscularis propria demonstrates late enhancement on the venous and interstitial phase DCE images [5, 14, 22].

Intermediate to low signal of the tumor on T2-weighted TSE images is seen compared to background hypointense muscularis propria and therefore could be differentiated from the normal bladder wall [5, 14, 22]. High DWI signal intensity of the tumor with corresponding low signal intensity on ADC compared to the muscularis propria [5, 14, 22] differentiates the tumor from the bladder wall. Prominent enhancement of the tumor compared to nonenhancing or minimally enhancing muscularis propria on the arterial phase of DCE imaging differentiates the tumor from the bladder wall [5, 14, 22].

These features help the recognition and staging of the tumor with differential signal compared to the underlying background bladder wall [5, 14, 22]. The extension of tumor into the muscularis propria and extravesical fat tissue differentiates T1 versus T2 and T2 versus T3, respectively [5, 14, 22].

A fibrotic and/or inflammatory stalk arising from the submucosa, with no evidence of malignancy, is usually associated with Ta and T1 tumors [5, 14, 22]. The stalk usually shows intermediate to low signal intensity on T2 TSE images although the signal can be variable [5, 14, 22]. Low signal on high b values images with

associated high signal on ADC map without diffusion restriction is also seen at the stalk which also usually shows early enhancement on DCE similar to the tumor [5, 14, 22]. The tumor is usually a non-muscle-invasive tumor and the tumor signal does not extend to the muscularis propria when the stalk is present [5, 14, 22]. Additional findings which are suggestive of non-muscle-invasive tumor also include tenting of the bladder wall and uninterrupted submucosal enhancement just beneath the tumor [5, 14, 22]. The submucosa sometimes is seen as a thickened layer under the tumor and the absence of diffusion restriction would be suggestive of inflammation and /or fibrosis. However, the stalk may occasionally show diffusion restriction without evidence of malignancy, and T1 tumors may also invade the submucosa without the presence of stalk [5, 14, 22]. Additionally, if there is discordance between the findings of T2 TSE, DWI or DCE, DWI should be the dominant sequence in staging due to the potential to differentiate the tumor tissue from inflammation and/or fibrosis [14, 28].

When the tumor is confined to the bladder wall and the intermediate tumor signal does not extend through the dark signal of muscularis propria completely on T2-weighted TSE images, the tumor is T2 [5, 14, 22]. The tumor shows a high DWI signal with a corresponding low ADC signal, and increased arterial phase enhancement confined to the wall without any extension to perivesical fat [5, 14, 22]. However, if there is discordance between the findings of T2 TSE, DWI or DCE, DWI should be the dominant sequence in staging due to the potential to differentiate the tumor tissue from inflammation and/or fibrosis [5, 14, 22]. DWI should be the dominant sequence in staging due to the potential to differentiate the tumor tissue from inflammation and/or fibrosis when there is perivesical inflammation and fibrosis due to treatment or postprocedural changes which may demonstrate similar signal to the tumor on T2-weighted images or similar enhancement to the tumor on DCE [5, 14, 22].

A recently proposed system for the determination of muscle-invasive disease on MRI which is called Vesicle Imaging-Reporting and Data System (VI-RADS) has been reported to have high accuracy with sensitivity of 87–92% and specificity of 79–87% [5, 22, 24–27]. However, this system is at its early stages of development, and there are still very limited studies for the validation of this system in the literature. Therefore, more studies are needed to determine its specific role in the diagnostic algorithm. VI-RADS) also depends on the determination of tumor extension through the bladder wall on T2-weighted TSE, DWI and DCE sequences. If the tumor is less than 1 cm with no evidence of extension of intermediate soft tissue tumor signal on T2 TSE, a corresponding signal on DWI/ADC signal and early enhancement on DCE into the muscularis propria, VI-RADS category is 1, and the muscle invasion is highly unlikely [5, 22]. If the tumor is larger than 1 cm with no evidence of extension of intermediate soft tissue tumor signal on T2 TSE, corresponding signal on DWI /ADC signal and early enhancement on DCE into the muscularis propria, VI-RADS category is 2, and the muscle invasion is unlikely [5, 22]. If there is exophytic intraluminal tumor without stalk or sessile tumor without evidence of non-enhancing T2 high signal intensity inner lining and without disruption of muscularis propria, VI-RADS category is 3, and the muscle invasion is equivocal

[5, 22]. If there is evidence of interruption of normal signal intensity of muscularis propria with tumor extension on T2 TSE with associated corresponding abnormal DWI/ADC signal and early enhancement on DCE, VI-RADS category is 4, and the muscle invasion is likely [5, 22]. If there is evidence of complete interruption of normal signal intensity of muscularis propria with tumor extension into the perivesical fat on T2 TSE with associated corresponding abnormal DWI/ADC signal and early enhancement on DCE through the whole muscularis propria, VI-RADS category is 5, and the muscle invasion is likely [5, 22].

When there is an extension of the tumor to the perivesical fat with the intermediate tumor signal disrupting the muscularis propria and seen beyond the confines of bladder wall on T2-weighted TSE images, the tumor is T3 (Fig. 5.5) [5, 14, 22]. High DWI signal with corresponding low ADC signal and increased enhancement of the tumor on the arterial phase extending into the perivesical fat beyond the confines of the bladder wall are also seen with T3 tumors [5, 14, 22]. Minimal to mild extension into the perivesical fat could still be present histopathologically, when the tumor involves the whole bladder wall without definite spread into the perivesical fat on MRI [5, 14, 22].

Invasion of the adjacent organs including the prostate, uterus, vagina and pelvic sidewalls, and abdominal wall represents T4 tumor (Fig. 5.6). T2 TSE is the dominant sequence for the evaluation of invasion of adjacent organs, and DWI and DCE are the adjunct sequences [14].

The detection of small tumors including the small flat or sessile lesions which are usually Tis, or tumors less than 1 cm is limited by MRI [14].

Variable signal intensity changes can be seen on T2-weighted TSE images including high-, intermediate-, and low-signal changes representing edema, inflammation, and fibrosis following post-biopsy and posttreatment changes [5, 14, 22]. Since these changes usually do not demonstrate diffusion restriction but show either high to intermediate signal on DWI and ADC map or low to intermediate signal on DWI and ADC map, DWI can be helpful for the differentiation of post-biopsy and posttreatment changes from the tumor [5, 14, 22].

N Staging

Internal iliac, external iliac, obturator, and presacral lymph nodes can be involved with N1-N2 lymph node-positive bladder cancer [15]. The common iliac chain lymph nodes are involved in N3 lymph node-positive bladder cancer [15]. More extensive retroperitoneal lymph node involvement above the level of common iliac chains is regarded as distant metastatic disease and staged as M1 [15].

The sensitivity and specificity of MRI including DWI in the identification of involved lymph nodes are 56–79% and 79–94% demonstrating limited accuracy of MRI [14, 29]. The presence of micrometastatic disease in lymph nodes equal to or smaller than 8–10 mm with normal morphology and lack of obvious diffusion restriction is a significant limitation leading to false-negative results [29–32]. Reactive and inflammatory changes of the lymph nodes mimicking metastatic lymph nodes are the other significant limitation leading to false-positive results due

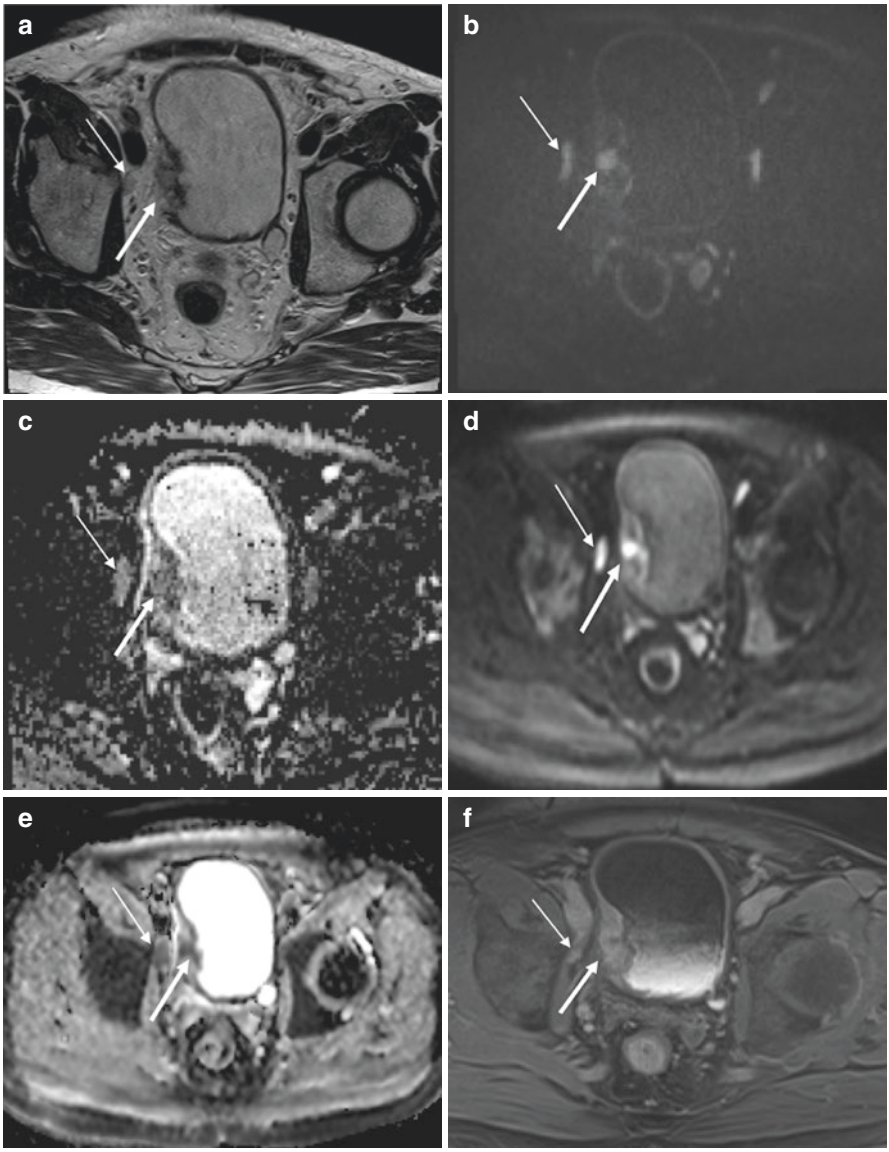

Fig. 5.5 Bladder cancer. Transverse T2-weighted high resolution turbo spin echo image (**a**), transverse high resolution, small field of view diffusion weighted image (**b**) and its corresponding ADC map (**c**), transverse standard resolution, large field of view diffusion weighted image (**d**) and its corresponding ADC map (**e**), and transverse T1-weighted fat-suppressed postgadolinium excretory phase three-dimensional gradient echo (**f**) image show enhancing bladder mass with irregular contours and associated diffusion restriction along the right bladder wall (thick arrows, **a**–**f**). The mass extends all the way through the bladder wall to the perivesicle fat, which is suggestive of T3 tumor. Please note the presence of bilateral pelvic sidewall prominent lymph nodes (thin arrows, **a**–**f**). This tumor is classified as VI-RADS 5 tumor

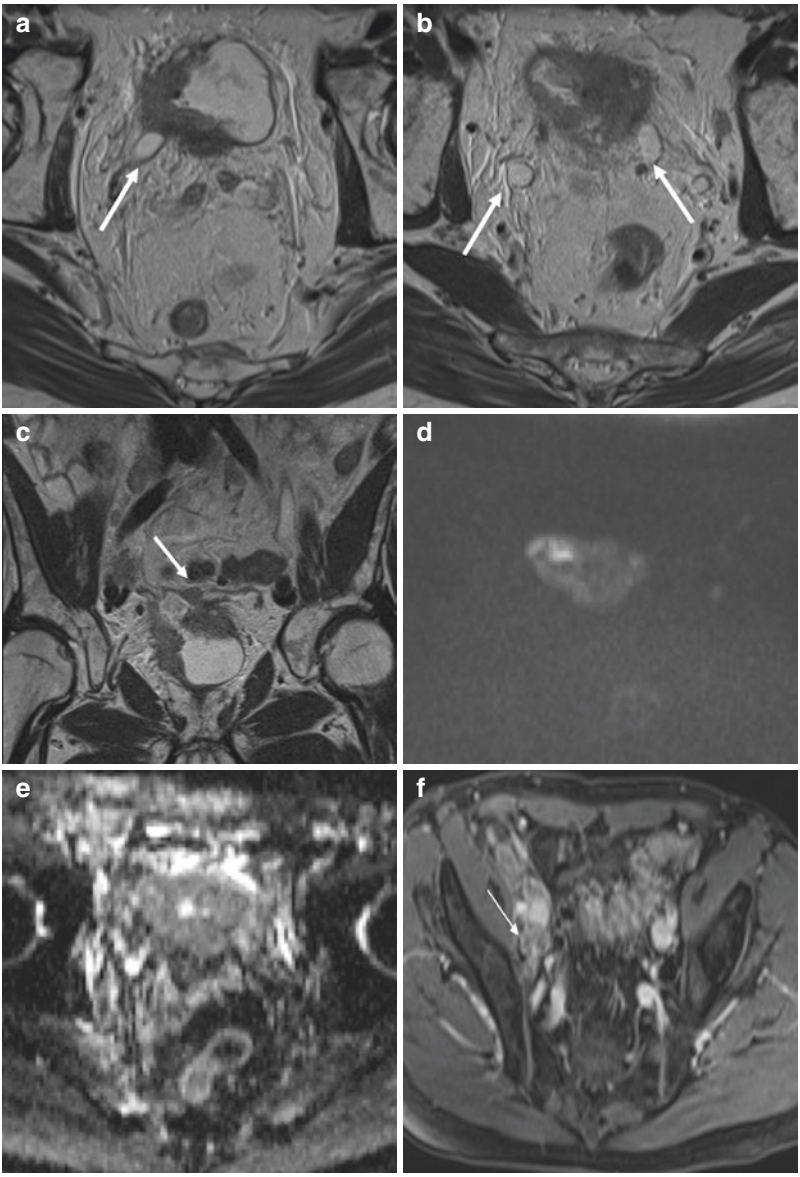

Fig. 5.6 Bladder cancer. Transverse (**a**, **b**) and coronal (**c**) T2-weighted high resolution turbo spin echo images, transverse diffusion weighted image (**d**) and its corresponding ADC map (**e**), and transverse T1-weighted fat-suppressed postgadolinium interstitial phase three-dimensional gradient echo (**f**) image demonstrate a large irregular enhancing bladder mass with associated diffusion restriction involving the bladder dome, bilateral lateral walls, and trigone. The mass involves the right (arrow, **a**) and the left (arrow, **b**) ureterovesicle junctions with associated hydroureters (arrows, **a**, **b**). The mass involves the peritoneum (arrow, **c**) between the bladder dome and sigmoid colon, and this is suggestive of T4 tumor. There are also multiple centrally necrotic enlarged lymph nodes along the right-sided iliac chains (arrow, **f**). This tumor is classified as VI-RADS 5 tumor

to overlapping MRI features including but not limited to size increase, increased enhancement, or obvious diffusion restriction [29–32].

However, diffusely increased heterogeneous T2 signal, focally increased homogeneous or heterogeneous T2 signal, focal or diffuse diffusion restriction, increased enhancement including focal or diffuse heterogeneous enhancement, asymmetrical increased cortical thickness, and asymmetrical shape compared to remaining lymph nodes or remaining part of the background normal lymph node architecture could be a clue for the diagnosis of lymph node involvement although none of these findings are specific.

Ultrasmall particle superparamagnetic iron oxide (USPIO) has the potential to identify metastatic involved lymph nodes) measuring less than 8–10 mm, which were otherwise could not be identified based on conventional size criteria [29, 32, 33]. These agents were taken by macrophages in normal lymph nodes, and lymph nodes showing benign inflammatory changes [29, 32, 33] demonstrating decreased T2 signal and appearing hypointense on T2 or T2*-weighted sequences [29, 32, 33]. However, metastatic lymph nodes are expected to show increased T2 signal and appear hyperintense on T2 or T2*-weighted sequences due to the lack of uptake by macrophages [29, 32, 33]. The sensitivity and specificity of USPIO for nodal staging in bladder cancer have been reported to be variable: 55–96% and 71–95% [14, 29, 32, 33], which could be due to false-negative results secondary to micrometastases in lymph nodes or false-positive results secondary to reactive hyperplasia, nodal lipomatosis, and insufficient uptake of USPIO.

Radiomics and Bladder Cancer

Radiomics is a developing translational field of imaging trying to find associations between extracted quantitative information obtained from imaging studies and clinical, laboratory, or histopathologic data with or without associated gene expression [6, 34, 35]. Quantitative information is extracted and analyzed by dedicated software, and this process is affected by image acquisition, postprocessing, and segmentation [6, 34, 35].

Quantitative features include shape features, first-order statistics, second-order statistics, and high-order statistics [6, 34, 35]. Shape features include dimensions, volume measurements or compactness, or surface features of tumors. First-order statistics use histogram-based features analyzing the intensities of voxels, their skewness (asymmetry), kurtosis (flatness), uniformity, and randomness (entropy) regardless of their spatial associations. Second-order statistics use textural features based on the interrelationships between adjacent voxels providing information on the spatial association of voxel intensities and lesion heterogeneity. High-order statistics use statistical methods to recognize any specific patterns, to suppress noise or to highlight details. Evaluation of these features can be enhanced by machine-learning techniques.

The role of radiomics analysis and features in the diagnosis and assessment of tumor response has not been still determined due to lack of sufficient data in the literature and limitations of this technique [34, 35]. The specific roles of individual parameters have also not been determined yet [34, 35]. A significant limitation for the use of radiomics features is the inability to have reproducible robust results without variability due to the dependence on acquisition technique and parameters [34]. Since the acquisition technique and parameters are very heterogeneous in the routine clinical practice, the radiomics analysis and its results are overall significantly and adversely affected [34]. Even the same studies done in the same scanner with the same parameters may end up giving different results depending on patient factors, contrast injection or enhancement changes, or scanning factors such as magnetic field inhomogeneities on MRI or imaging artifacts [34, 35]. Additionally, the lack of fully automated postprocessing and segmentation techniques with high accuracy also leads to variable results with low reproducibility [34]. Therefore, it is essential to describe the most useful radiomics parameters, define their roles in the diagnosis of tumors and assessment of treatment response, and determine the optimal ways to have reproducible robust results with the use of standard acquisition techniques and highly accurate fully automated postprocessing and segmentation techniques.

A limited number of studies on radiomics of bladder cancer have been reported in the literature. These initial studies used radiomics features for the staging of bladder cancer and assessing treatment response. Some specific studies demonstrated that radiomics features may play a role and may have high accuracy in the determination of tumor recurrence following TURBT [36], pathologic grade of tumor based on MRI [37], the muscle-invasive status of the tumor or extension of tumor to perivesical fat [38], and tumor volume changes following treatment [39, 40]. Due to limited number of studies, more research studies are needed to determine the role of radiomics in the assessment of bladder cancer.

Conclusion and Future Directions

Although imaging is essential for the diagnosis and staging of bladder cancer and assessment of tumor response, it is still limited, and therefore, direct visualization with cystoscopy and histopathologic assessment are the preferred standard methods for initial diagnosis and assessment of bladder wall involvement. High-spatial-resolution MR imaging of bladder cancer could be a promising technique for bladder cancer staging although its specific role has not been determined yet and this technique still needs validation. However, MR imaging techniques not only using high spatial resolution but also using higher contrast resolution in combination with motion resistant techniques may have higher potential to diagnose and stage bladder cancer and assess treatment response. Dual-source CT techniques by using different photon energies should also be studied for the assessment of bladder cancer. Radiomics features may also increase the accuracy of bladder cancer assessment

and could be a helpful feature in radiologic assessments. Hybrid imaging techniques employing PET and pharmaceuticals are also promising and will be discussed in Chap. 7.

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Chapter 6
Evaluation of Hematuria

Ashley N. Gonzalez and Richard S. Matulewicz

Epidemiology and Definitions of Hematuria

Hematuria has long been recognized as a potential harbinger of serious urologic pathology and is one of the most common reasons for urologic referral and evaluation. Since there is a significant range of potential benign and malignant etiologies to hematuria, some form of evaluation is generally recommended. However, as our understanding of risk factors for disease as well as the natural history of patients who present with hematuria has grown, the evaluation paradigm for hematuria has changed significantly over recent decades and continues to evolve.

Hematuria is thought to exist on a continuum of severity but is traditionally classified as either gross (visible to the eye) or microscopic (detected via a laboratory test such as a urinalysis). Depending on the degree of hematuria, many different medical specialty or society guidelines describe distinctly different evaluation approaches. Microscopic hematuria (MH) is most strictly defined by the presence of red blood cells seen under high-powered microscopy and is quantified as the number per high-power field (RBCs/HPF) on microscopic analysis. Though the threshold for "clinically significant" microscopic hematuria varies, the American Urologic Association (AUA) number that should initiate an evaluation has been most recently defined as 3 or more RBCs/HPF [1]. Historically, clinically significant microscopic hematuria has also been defined by multiple "positive" urinalyses (≥3 RBC/hpf) or based on qualitative urine

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dipstick assays. There is controversy regarding the necessity of a confirmatory microscopic urinalysis when a dipstick assay is used, but this practice is currently recommended by some guideline panels. In addition to meeting the RBC/HPF threshold, clinically significant MH requiring an evaluation is traditionally MH that is present in "the absence of an obvious benign cause." This definition is common among several guideline recommendations, including the current AUA guidelines, and can exclude patients with a urinary tract infection, known medical renal disease, or any other confirmed causative etiology [1].

Routine screening urinalysis (UA) is not currently recommended by the United States Preventative Services Task Force (USPSTF), AUA, or any academic organization for the detection of underlying GU malignancies. However, urinalysis is a commonly used laboratory test to help in managing many other common health problems including medical renal disease, diabetes, and urinary tract infection. Therefore, urinalyses are frequently obtained by primary care providers (PCPs), and these incidental findings of microscopic hematuria account for the vast majority of subsequent urologic referrals. Older screening studies of various populations have shown that MH is a relatively common finding, ranging in prevalence from 4% to 21% depending greatly on the population under investigation and the screening methodology (Table 6.1) [2–4].

Gross hematuria (GH) is defined as visible blood in the urine and can range from thin, pink-tinged urine to dark, thick blood with clots. The quality of gross hematuria can give insight into the location of bleeding: the presence of clots generally indicates non-glomerular bleeding; large, thick clots suggest bleeding from the bladder; and small, stringy clots may suggest upper tract bleeding [5]. Further, the timing of hematuria during the urinary stream may also provide insight into the source of bleeding. Gross hematuria exclusively at the start of urination suggests a distal urethral etiology, whereas terminal hematuria may suggest bladder neck or prostatic bleeding, and hematuria throughout the urinary stream indicates bleeding from the bladder or upper urinary tract [5].

Etiologies of Hematuria

Gross and microscopic hematuria have numerous potential etiologies of benign and malignant origin (Table 6.2). A causative factor can be identified in a majority of hematuria cases, though an idiopathic cause is seen in upwards of 40% with microscopic hematuria [6].

Table 6.1 Prevalence of MH in various sample populations

Patient characteristics	Means of detection	Prevalence of AMH
Men over 50 [2]	Positive dipstick UA	21.1%
Men over 40 [3]	≥1 RBC/HPF	4%
Men over 35 and post-menopausal women [4]	≥1 RBC/HPF	13%

Table 6.2 Causes of hematuria

Pseudohematuria	Benign	Malignant
Myoglobinuria	Nephrolithiasis	Urothelial neoplasm (including bladder and upper tract)
Betadine contamination	Renal cyst	Renal mass
Bloody semen	Urinary tract infection	Prostate cancer
Vaginal bleeding	Sexually transmitted infection	Metastases
Beeturia	Benign prostatic enlargement	Malignant obstruction
	Radiation cystitis	
	Interstitial cystitis	
	Intrinsic renal disease	
	Recent urologic instrumentation (including Foley catheter)	
	Urologic trauma	
	Urethral stricture	
	Rhabdomyolysis/strenuous exercise	
	Idiopathic	

Pseudohematuria

Prior to initiating an evaluation for hematuria, an accurate diagnosis should first be made. A reported history of gross hematuria is sufficient for the initial further workup, given the intermittency of hematuria and the higher risk of an underlying malignancy. However, since dipstick urinalyses are commonly used in the primary care setting, the current recommendation is to perform a confirmatory microscopic urinalysis when microscopic hematuria is detected qualitatively. Although the presence of microscopic hematuria on dipstick urinalysis is strongly correlated with microscopic analysis, its inferior sensitivity has maintained microscopic analysis as the gold standard for detection of MH [7, 8]. The inability of dipstick urinalysis to distinguish hemoglobin from myoglobin may account for false positives of MH in the right clinical setting. Betadine from urethral preparation prior to collection may also result in a false-positive dipstick UA [9]. Other false positives unrelated to testing assays include postcoital urine studies in men and vaginal bleeding in women [10]. Women who present during menstruation or with vaginal bleeding may require urinary catheterization or repeat urine collection to confirm a diagnosis of hematuria prior to further testing.

Benign Causes of Hematuria

There are many potential benign causes of hematuria. In an otherwise healthy and young patient, urinary tract infections and nephrolithiasis are among the most common causes of hematuria, while benign prostatic enlargement (BPE) is the most

common cause in older men. Often, the clinical history and physical exam will offer insight into the etiology of hematuria. However, in patients with risk factors such as more advanced age or those with a smoking or exposure history, benign etiologies should be a diagnosis of exclusion after ruling out the more serious and life-threatening malignant etiologies with a thorough urologic evaluation.

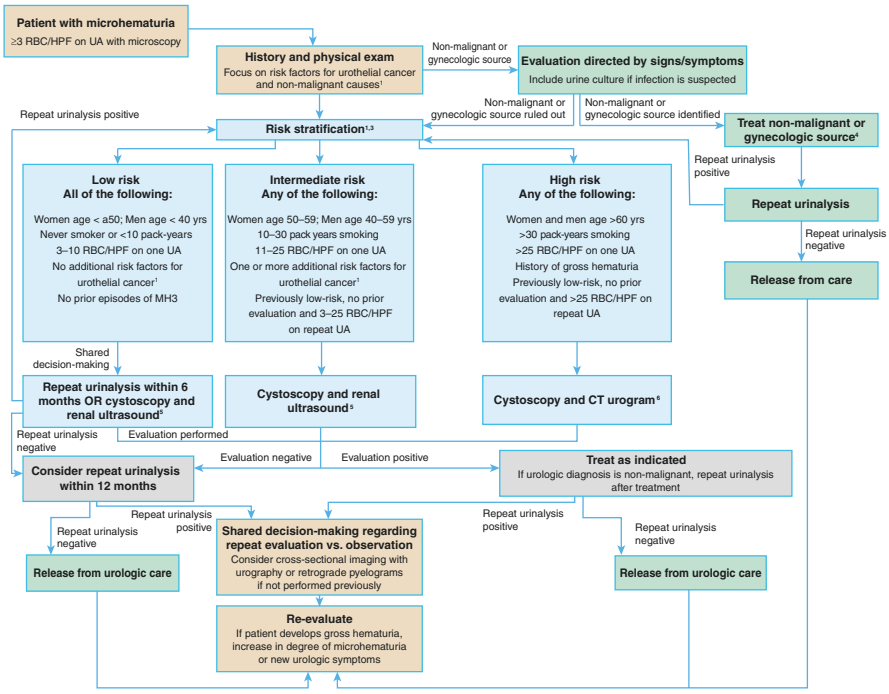
Malignant Causes of Hematuria

The more concerning etiologies of hematuria are those that represent underlying malignancies of the urinary tract. The prevalence of underlying urologic malignancies is related to the degree of hematuria (gross vs. microscopic hematuria). A 2020 meta-analysis found a pooled detection rate of 3.2% (0–16%) for bladder cancer, 0.042% (0–3.5%) for upper tract urothelial carcinoma, and 0.28% (0–9.7%) for kidney cancer [11]. It is important to note the wide ranges and heterogeneity that is present among the included studies that form the basis of this meta-analysis which highlights the differences in definitions of MH, inclusion/exclusion criteria, degree of urologic workup (including imaging modality), and study design. The prevalence of an underlying urologic malignancy in patients presenting with MH is low. In recent years, a better understanding of objective risk factors related to malignancy as well as the harms associated with overevaluation has led to an evolution in the diagnostic approach in order to limit low yield evaluations. Alternatively, little controversy exists regarding the need for a prompt urologic evaluation in anyone with gross hematuria since the incidence of urologic malignancies are much higher in these patients with cancer found in upwards of 20% [12–15].

Indications for Workup of Hematuria

AUA Guideline Review

Numerous international guidelines inform the urologic evaluation and management of patients with hematuria. In 2020 the AUA revised their microscopic hematuria guidelines, shifting from a universal evaluation strategy for all people with microscopic hematuria older than 35 to a risk-stratified and shared decision-making approach which spares the lowest risk individuals from invasive and extensive testing. Figure 6.1 displays the 2020 AUA algorithm for MH risk stratification and workup [1]. In the updated guideline recommendations, microscopic hematuria is still defined as three or more RBCs/HPF on microscopic urinalysis. The initial evaluation is recommended to include a focused history and physical exam which aims to identify known risk factors for GU malignancies (Table 6.3) and ruling out benign causes of MH (Table 6.2). If pseudohematuria or a benign cause for MH is identified



1. Main risk factors for urothelial cancer are those in the AUA risk stratification system (age, male sex, smoking, degree of microhematuria, and history of gross hematuria). Additional risk factor for urothelial carcinoma include but are not limited to irritative lower urinary tract voiding symptoms, history of cyclophosphamide or ifosfamide chemotherapy, family history of urothelial carcinoma or Lynch Syndrome, occupational exposures to benzene chemicals or aromatic amines, history of chronic indwelling foreign body in the urinary tract
2. If medical renal disease is suspected, consider nephrologic evaluation, but pursue concurrent risk-based urological evaluation
3. Patients may be low-risk at first presentation with microhematuria, but may only be considered intermediate-or high-risk if found to have persistent microhematuria
4. There are non-malignant and gynecologic sources of hematuria that do not require treatment and/or may confound the diagnosis of MH. Clinicians can consider catheterized urine specimen in women with vaginal atrophy or pelvic organ prolapse. Clinicians must use careful judgment and patient engagement to decide whether to pursue MH evaluation in the setting of chronic conditions that do not require treatment, such as the aforementioned gynecologic conditions, non-obstructing stones, or BPH
5. Clinician may perform cross-sectional imaging with urography or retrograde pyelograms if hematuria persists after negative renal ultrasound
6. MR Urogram or non-contrast imaging plus retrograde pyelograms if contraindications to CT Urogram

Fig. 6.1 AUA 2020 algorithm for asymptomatic microscopic hematuria. (Reproduced with permission from [1])

based on the initial history and physical exam, that cause should be addressed and a repeat UA performed thereafter to assess for resolution or persistence of hematuria which requires further evaluation. Patients are stratified as either low-, intermediate-, or high- risk. Low-risk patients are limited to women <50 and men <40, those patients with less than a 10-pack year smoking history, only 3–10 RBCs/HPF, no prior episodes of MH, and have no other risk factors for GU malignancies (Table 6.3). These low-risk patients are recommended to either undergo cystoscopy and renal ultrasound or may opt for repeating urinalysis within 6 months after shared decision-making. Those with persistent MH (on repeat urinalysis after 6 months) are considered intermediate risk which prompts an evaluation. Intermediate-risk patients also include women aged 50–59 and men aged 40–59, those with a 10–30 pack year smoking history, those with 11–25 RBCs/HPF, or those with at least one additional risk factor for GU malignancies. Intermediate-risk patients are recommended to undergo a cystoscopy and renal ultrasound. Patients are considered to be of high risk if they meet any of the following criteria: age >60, >30 pack year smoking

Table 6.3 Urothelial cancer risk factors

Risk factors included in AUA MH risk stratification algorithm	
	Age
	Male gender
	Smoking history
	Degree of microscopic hematuria
	Persistence of microscopic hematuria
	History of gross hematuria
Additional risk factors described though not included in AUA algorithm	
	History of pelvic radiation
	History of urothelial malignancy
	Chronic indwelling Foley
	Irritative lower urinary tract symptoms
	Schistosoma haematobium infection
	HPV infection
	Cyclophosphamide use
	Bladder stones
	Chronic cystitis
	Occupational exposures to aromatic amines, dyes, rubbers, textiles, paints, leather, chemicals

history, >25 RBCs/HPF, or a history of gross hematuria. High-risk patients are recommended to undergo cystoscopy and CT urogram. Of note, per AUA guidelines urine cytology should not be used in the initial evaluation of patients with MH, though may be obtained in the setting of persistent MH after a negative workup or irritative voiding symptoms or risk factors for carcinoma in situ (CIS) [1].

Although there is no specific AUA guideline for gross hematuria, gross hematuria should be evaluated with cystoscopy, CT urogram, and urine cytology. Gross hematuria in older patients who have had recent instrumentation (including Foley catheter placement) or those on anticoagulation but who also have risk factors for GU malignancies represent a challenging population though full workup is generally indicated. Use of anticoagulation medications has been shown to increase the risk of hematuria and also the detection of bladder cancers [16].

Variation Among International Hematuria Guidelines

There is considerable variation among international guidelines for the diagnosis and management of hematuria. While the majority of this chapter will reflect upon the AUA guidelines, the variability across organizations is important to note as it highlights the challenges of managing these patients and the nuances in various treatment approaches (Table 6.4).

Table 6.4 International hematuria guidelines

Guideline	Microscopic hematuria definition	Age for evaluation of MH	Cystoscopy	Upper tract imaging	Cytology	Follow-up	Gross hematuria workup
AUA 2020 [1]	≥3 RBCs/HPF	Risk stratified starting at age 40 for men and 50 for women	Indicated for most patients through risk-stratified approach and shared decision-making	None vs r renal ultrasound vs CT urogram based on risk and shared decision-making	Not recommended in the initial evaluation for AMH Consider in repeat evaluations for AMH or in patients with irritative voiding symptoms	Repeat UA after treating nonmalignant cause or in 1 year	No specific recommendations though cystoscopy, CTU, and urine cytology generally indicated
NICE [17]	No specific criteria for a number of RBCs/HPF though patient must also have dysuria and elevated serum WBC	≥45	No specific workup is detailed, simply a referral to urology				Referral for workup if aged >45
Canadian Consensus [18]	≥3 RBCs/HPF	≥35 for AMH All patients at any age with symptomatic MH	All patients	CTU for symptomatic MH, Imaging modality for AMH at the discretion of the provider	Symptomatic MH, Yearly for 3 years following negative workup in patients with risk factors for urothelial cancer	Annual UA for 3 years Repeat evaluation	All patients at any age: Cystoscopy, CTU, and urine cytology

(continued)

Table 6.4 (continued)

Guideline	Microscopic hematuria definition	Age for evaluation of MH	Cystoscopy	Upper tract imaging	Cytology	Follow-up	Gross hematuria workup
American College of Obstetricians and Gynecologists (ACOG) [19]	Women aged 35–50 who are low-risk, never smokers with <25 RBCs/HPF should not undergo evaluation		No specific workup is detailed				
American College of Physicians [20]	≥3 RBCs/HPF	Not specified	No specific workup, details referral to urology				Any adult with GH should be referred to urology
JUA [21]	≥5 RBCs/HPF	>40	All men, smokers, or additional risk factors for urothelial malignancies	Ultrasound	Patients without risk factors for urothelial malignancies should get noninvasive cytology or ultrasound (no cystoscopy)	Retesting if LUTS, GH, or persistence	Vague but involves imaging (ultrasound, CTU, MRU) and cytology
BAUS/RA [22]	≥1+ on dipstick UA (on single for symptomatic MH, on 2/3 dipsticks for asymptomatic MH)	>40	All patients	All patients (imaging modality not specified)	Age >50 after a negative evaluation	Re-referral to urology if LUTs or GH develop	All patients with GH should be referred to urology for cystoscopy, imaging, and cytology

Patient History and Unique Patient Circumstances

A careful, focused history and physical exam is the single most important aspect of the initial evaluation for patients presenting with hematuria. Unfortunately, given the prevalence of hematuria (especially microscopic hematuria) observed in the primary care provider's office, several studies have shown that urologic referrals for both gross and microscopic hematuria are often delayed [23–25]. Several patient cohorts in particular represent those who may present in a delayed manner.

Women

Although bladder cancer is up to four times more common in men than women, women are more likely to be diagnosed at a later stage and have a disproportionately high mortality from bladder cancer. This gender disparity has widely been attributed at least in part to delays in diagnosis, namely, the recognition of hematuria as a serious sign of indolent urologic pathology [24, 26]. In particular, women are more likely to undergo symptomatic treatments for hematuria and concomitant irritative voiding symptoms than men, often involving antibiotic therapy for confirmed or presumed urinary tract infections [27]. Women, therefore, must be followed closely by primary care providers, and there must be careful consideration for urologic referral for further workup if symptoms and hematuria persist despite initial management.

Antithrombotics

The AUA guidelines make a specific reference regarding patients taking antiplatelets and anticoagulants, stating that these patients should undergo the same evaluation as those patients not on such agents [1]. All antiplatelets (including aspirin, clopidogrel, prasugrel, ticagrelor, ticlopidine, dipyridamole) and anticoagulants (including warfarin, apixaban, rivaroxaban, dabigatran) have been shown to significantly increase rates of hematuria-related complications [16]. Of note, patients exposed to antithrombotics had a higher likelihood of being diagnosed with bladder cancer than those unexposed, even after adjusting for age and sex (standardized incidence ratio 2.38, 95% CI 2.23–2.44) [16]. While antithrombotics may exacerbate hematuria from benign causes, they may also unmask indolent bladder cancers. Hematuria to any degree must not be attributed to the administration of these agents without a complete urologic evaluation.

Components of the Diagnostic Evaluation of Hematuria

A complete hematuria workup has several components that aim to fully evaluate the entire genitourinary tract. The two primary aspects are upper tract imaging and visual inspection of the bladder with cystoscopy. The most commonly used imaging modality is computed tomography either with or without contrast. CT scans can be protocoled to evaluate the urinary tract in several ways including a "urogram," which is both a non-contrast and timed to enhance the renal parenchyma as well as opacify the drainage system, or a triphasic scan which includes a non-contrast phase to assess for nephrolithiasis, an arterial phase to assess for enhancement of renal cortical masses, and an excretory phase to assess the drainage system. Other modalities include ultrasonography or magnetic resonance imaging (MRI). MRIs can similarly be protocoled and enhanced with contrast as CT scans and may be used in patients with poorer renal function who are unable to get iodinated contrast dye. Cystoscopy represents the gold standard for complete evaluation of the bladder, though noninvasive modalities have been and are currently being studied. Urine cytology and urinary biomarkers represent an emerging adjunct with specific indications in the workup of hematuria.

Cystoscopy

Since the earliest iteration in the 1800s, cystoscopy has continuously evolved over centuries to become an invaluable tool to the urologist [28]. Though cross-sectional imaging is a useful adjunct for gaining a glimpse into the bladder, cystoscopy remains the gold standard for diagnosing intravesical pathology, namely, bladder cancer, and is a critical component of the workup for hematuria. Per AUA guidelines, cystoscopy is recommended in all intermediate and high-risk patients with MH and in all low-risk patients who pursue further workup through shared decision-making, as well as in all patients with a history of gross hematuria. In contrast to standard white light cystoscopy, recent innovations in cystoscopic evaluation include blue-light cystoscopy with hexaminolevulinate which preferentially accumulates and fluoresces in neoplastic tissue [29]. Similarly, narrow band imaging offers an alternative enhanced endoscopic technique in which different wavelengths of light are used that are strongly absorbed by hemoglobin to offer improved contrast of superficial tumors from the bladder wall [30]. Each of these methods (white light, blue light, narrow band imaging) has its advantages and disadvantages, and recent work by Kriegmair et al. sought to utilize the various imaging modalities to create a novel multiparametic cystoscopy in real time that as a feasibility study shows promise for future work [31, 32]. In addition to traditional cystoscopy, advances in imaging techniques have led to the development of virtual cystoscopy in which cross-sectional imaging is reconstructed to produce a three-dimensional

model of the bladder. While this technique has not made it into any guidelines for the evaluation of hematuria, it is a potential adjunct in patients at high risk for bladder cancer who refuse cystoscopy [33, 34]. While only white light cystoscopy is currently recommended by AUA guidelines for the initial evaluation of hematuria, there are an ever-growing number of clinically available tools that aim to enhance our diagnosis and management of patients with bladder cancer.

Upper Tract Imaging

The optimal use of upper tract imaging in the initial evaluation of hematuria is controversial. The accuracy (sensitivity, specificity, and predictive value) of each modality must be balanced with the cost to the healthcare system and risk to the patient. Greater recognition of these factors has been a major driving force towards more prudent use of CT scans with ionizing radiation in the updated 2020 AUA guidelines.

Cross-Sectional Imaging with Contrast Enhancement

Since the introduction of CT to clinical practice in 1973, the rapid advancement of technology, data acquisition, and image processing has rapidly evolved to become an invaluable tool. CT urography (CTU) involves an intravenous contrast study imaging the kidneys, ureters, and bladder with delayed phase imaging during the excretory phase to fully evaluate the GU tract for pathology, in particular filling defects that may indicate an underlying urothelial malignancy. While unenhanced or non-urographic cross-sectional studies such as renal ultrasound and non-contrast CT have high diagnostic accuracy for stones and renal masses, they incompletely visualize the urothelial lining of the urinary tract. CTU has a sensitivity between 0.818 and 0.970 and specificity between 0.930 and 0.998 for upper tract lesions based on recent literature [35–37]. For patients with contraindications to CTU (commonly due to impaired kidney function and intravenous contrast load), MR urogram (MRU) is an alternative per AUA guidelines. While not recommended as the first-line imaging modality in the evaluation of patients with hematuria, several studies have sought to evaluate the diagnostic accuracy of MRU for evaluating the upper urinary tract with overall excellent results [38–40]. Sudah et al. reported their findings in a prospective study evaluating 29 patients and found that all upper tract lesions were identified by both modalities [38–40]. A major consideration for the use of MR and CT as well for the evaluation of hematuria is the cost of such evaluations as well as the diagnostic yield of such extensive evaluations.

Renal Ultrasound

Prior to the updated 2020 AUA guidelines, CTU was recommended as part of the workup for all patients with microscopic hematuria (essentially all patients over age 35); however, with the updated risk-stratified approach, renal ultrasound is now recommended as opposed to CTU for the initial workup of microscopic hematuria in low- and intermediate-risk patients (Fig. 6.1). While renal ultrasound has a lower sensitivity and specificity than CTU in detecting upper tract lesions (0.56–0.96 and 0.940–1.00, respectively), it remains a reasonable option in the initial evaluation for lower-risk patients [41, 42]. In their decision analytic model comparing four different diagnostic strategies (CTU and cystoscopy, renal ultrasound and cystoscopy, cystoscopy alone, and CTU alone), Halpern et al. examined the costs and effectiveness of each of these modalities for the initial investigation of MH [43]. They found that ultrasound and cystoscopy were the most cost-effective approach and that replacing ultrasound with CTU detected only one additional cancer per 10,000 patients at a cost of almost 6.5 million dollars [43].

Urine Cytology

Since its first description for the detection of urothelial malignancies in 1945, the application of urine cytology to the detection and follow-up of patients with urothelial malignancies has evolved immensely [44]. As Papanicolaou wrote, "One would expect that in a cancerous lesion of one of the urinary organs, superficial cancer cells would become exfoliated into the excretory ducts and be carried out by the urine." [44] Urine cytology now plays a valuable role in the long-term surveillance of bladder cancer and UTUC, with an overall sensitivity and specificity of 0.20–0.53 and 0.83–0.997, respectively [45]. Importantly, urine cytology has been shown to have consistently higher sensitivities for high-grade tumors and CIS that sharply declines in patients with low-grade tumors [46]. This limitation underlies the broad use of urine cytology in higher-risk patients with GH and the AUA recommendations against urine cytology in the initial evaluation of MH [1]. However, the AUA does support its use in patients with persistent MH after a negative evaluation or in patients with irritative voiding symptoms which may suggest carcinoma in situ (CIS) [1]. Urine cytology finds more consistent use in the surveillance and management of known urothelial malignancies and is included in the corresponding NCCN and AUA guidelines [47–49].

Future Potential Use of Urine Markers and Biomarkers

While cytology remains the oldest and most widely used noninvasive urinary test for urothelial malignancies, its limited sensitivity has led to the investigation of many other protein- and gene-based biomarkers (Table 6.5). The majority of these

biomarker studies were conducted in the setting of surveillance for urothelial malignancies with promising results, and their translation to the primary evaluation of hematuria remains an active area of interest. Sathianathen et al. conducted a systematic review and meta-analysis involving the Food and Drug Administration (FDA)-approved AssureMDx, bladder tumor-associated antigen (BTA), CxBladder, NMP22, UroVysion, and ImmunoCyt/uCyt+ in the evaluation of primary hematuria [50]. These data are included in Table 6.5 and are compared to the overall performance of those biomarkers. AssureMDx had the highest sensitivity and specificity in the primary evaluation of hematuria, studied in a total of 354 patients. Additional studies have explored the role of these biomarker assays in the context of selecting patients who should undergo cystoscopy [51, 52]. In their validation study, vanKessel et al. report that AssureMDx had a negative predictive value of 99% and could lead to a 77–82% reduction in cystoscopies for the primary evaluation of hematuria at a cost of just $23 per patient compared to $627 for a negative cystoscopy [52]. Despite these promising results for adjunct noninvasive testing that may change the clinical landscape for the detection of urothelial malignancies, cytology remains the only urinary marker currently recommended by the AUA as part of the evaluation for microscopic or gross hematuria given the overall limited data [1].

Table 6.5 Select urinary biomarkers for detection of urothelial carcinoma and their diagnostic performance in the setting of hematuria

Biomarker	Marker detected	Sensitivity (primary hematuria meta-analysis) [50]	Specificity (primary hematuria meta-analysis) [50]
BTA stat	Complement factor H-related protein (qualitative)	0.67 (0.40–0.85)	0.68 (0.55–0.79)
BTA TRAK	Complement factor H (quantitative)	–	–
ImmunoCyt/uCyt+	Fluorescent antibodies directed against mucins	0.83 (0.78–0.0.87)	0.87 (0.85–0.89)
NMP22 quantitative	Nuclear matrix protein	0.79 (0.63–0.90)	0.76 (0.67–0.93)
NMP22 qualitative	Nuclear matrix protein	0.70 (0.46–0.87)	0.85 (0.83–0.87)
UroVysion	Fluorescence in situ hybridization assay detecting aneuploidy in chromosomes 3,7, 17, and loss of 9p21	0.69 (0.55–0.80)	0.78 (0.75–0.83)
CxBladder	MDK, HOXA13, CDC2, IFGBP5, CXCR2	0.82 (0.71–0.89)	0.85 (0.81–0.88)
Assure MDx	Oncogene mutation assay including FGFR3, TERT, HRAS, OTX1, ONECUT2, TWIST1	0.95 (0.87–0.98)	0.85 (0.79–0.89)

Risks Associated with Evaluation

The diagnosis of occult urologic malignancies relies on the careful evaluation of all patients presenting with hematuria. The optimal evaluation has yet to be determined, though a risk-stratified approach as reflected in the 2020 AUA guidelines is an important step towards avoiding low value and potentially harmful care associated with overevaluation. Georgieva et al. created a microsimulation model of the costs and harms associated with various international guideline recommendations in the evaluation of MH [53]. The prior 2012 AUA guidelines were included as a comparator which recommended cystoscopy and CTU for all patients over age 35. Dysuria, UTI, false positives, contrast allergies, contrast nephropathy, radiation-induced cancers are all important clinical outcomes that must be weighed against the risk of missing a cancer during the initial evaluation [53]. The costs of hematuria evaluations have been well-described and present a potentially unnecessary burden to the health care system if there were improved methods of minimizing workups without sacrificing diagnostic yield [43, 53].

Novel Risk Stratification Approaches for Microscopic Hematuria

There is little debate that gross hematuria, given the high risk of associated urologic malignancy should be fully worked up with cystoscopy, CTU, and consideration of urine cytology. The optimal workup of patients with MH has yet to be defined, though many authors have sought to design and implement risk stratification models that go beyond the AUA's model to more accurately predict the risk of urologic malignancies. One such risk stratification tool is the hematuria risk index (HRI) initially described in 2013 in a prospective cohort study involving 2630 patients who were referred to urologists with AMH and underwent a complete workup including CTU and cystoscopy [54]. The resulting HRI assigned a total number of points ranging from 0 to 11; 4 points were given for a history of GH or age over 50 years, and 1 point was given for a history of smoking, male gender, and greater than 25 RBCs/HPF on urinalysis [54]. Patients were then stratified into low- (0–4 points), intermediate- (5–8 points), and high-risk (9–11 points) groups with corresponding cancer detection rates of 0.3%, 1.1%, and 11.6%, respectively [54]. The initial validation cohort performed similarly well with an area under the curve of 0.829 [54]. This model was the basis for a retrospective study involving 1049 patients who underwent evaluation for AMH, focusing on the low- and intermediate-risk groups as patients with gross hematuria were excluded [55]. In this cohort, malignancies were detected in 0% of low-risk and 2.96% of intermediate-risk patients [55]. The authors went on to look at the cost-effectiveness of implementing

the HRI in practice and found that omitting the low-risk cohort in whom no malignancies were detected (and only 0.3% detection rate in prior study) would result in a 60% cost savings ($408,376 to $166,252) [55].

Additional attempts at risk stratification using nomograms have been undertaken. The benefit of this approach is a more personalized risk assessment based on individual factors rather than generalized risk strata [56]. Nomograms assign a point total that corresponds to categories or values of risk factors that may include increasing age, degree of hematuria, male gender, smoking status, and race [56, 57]. One such model involving a training cohort of 2126 patients with MH performed well with a negative predictive value of 99.7 for bladder cancer and would lead to only 1 missed diagnosis of bladder cancer while avoiding 335 if no patients below the 0.01 probability underwent workup [56]. A second model which also included patients with gross hematuria and incorporated cytology findings produced a nomogram that achieved 83.1% accuracy in predicting the presence of bladder cancer and provides a useful tool to use when discussing risks with individual patients [57].

The 2020 AUA guidelines for microscopic hematuria provide significant changes from prior iterations, aiming to reduce the use of low-value diagnostic evaluations without missing an opportunity to diagnosis indolent urothelial malignancies. Future validation studies are needed to evaluate whether the new guidelines accomplish this task. Initial work from Woldu et al. suggest that risk stratification into low-, intermediate-, and high-risk cohorts is highly correlated with the risk of bladder cancer (0.4%, 1.0%, and 6.3%, respectively) [58]. Furthermore, over 80% of the patients evaluated were in the high-risk category, suggesting what has been well-described previously in that primary care providers are already risk stratifying to some degree-independent of guidelines when deciding which patients to refer to urology [59]. This is a bias that plagues much of the existing literature on MH and inevitably overestimates the risks of urologic malignancies in the general population. Further prospective work that mitigates referral bias and incorporates all components of the AUA risk stratification model is necessary, and this work provides an important starting point.

Conclusion

There is much work to be done to optimize the investigation of patients with hematuria. Further incorporation and validation of risk stratification models, the potential for applying urinary biomarkers into stratification models, and additionally increasing primary care provider adherence to guidelines and referral practices all remain important areas for improving the critical early diagnosis and treatment of urothelial malignancies.

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Chapter 7
Molecular Imaging Modalities: Applications of Current and Novel Radiotracers

Chelsea K. Osterman and Tracy L. Rose

Introduction

Imaging plays a central role in the diagnosis and management of bladder cancer throughout the disease course, including the initial cancer staging, assessment of treatment response, and surveillance for disease recurrence. This has traditionally been performed using conventional imaging modalities such as computed tomography (CT) and magnetic resonance imaging (MRI).

More recently, these have been combined with molecular imaging techniques, such as positron-emission tomography (PET), to noninvasively evaluate cellular processes. Such techniques can provide both structural and functional information, creating a more detailed assessment of a patient's cancer status.

Several radiotracers have been developed for use in molecular imaging (Table 7.1), with many more under active investigation. These radiotracers vary in their mechanism of action and measured effect, with the potential to significantly impact disease management and improve patient outcomes. In this chapter, we review applications of current and investigational radiotracers for the diagnosis and management of bladder cancer.

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Table 7.1 Selected radiotracers with applications in patients with bladder cancer

Tracer	Mechanism	Measured effect	Advantages	Disadvantages
^{18}F-FDG	Glucose analog	Glucose metabolism	Widely available Increased familiarity with imaging interpretation High sensitivity in detecting osteolytic lesions	Limited evaluation of bladder tumor and local lymph nodes Uptake in inflammatory lesions Less sensitive to detect osteoblastic lesions
^{11}C-Choline	Precursor in phospholipid biosynthesis [24]	Cell proliferation	Minimal urinary excretion Low background radioactivity in the pelvis	Short half-life limits use to centers with onsite cyclotron Uptake in inflammatory lesions
^{11}C-Acetate	Substrate of β-oxidation for the synthesis of cholesterol and lipids	Fatty acid synthesis [19]	Minimal urinary excretion	Short half-life limits use to centers with onsite cyclotron Uptake in inflammatory lesions Accuracy negatively affected by prior BCG [20]
^{99m}Tc-MDP	Mimics endogenous pyrophosphate, which accumulates in bone to form hydroxylapatite [44]	Bone formation	Widely available High sensitivity in the detection of primarily osteoblastic lesions Low cost Used with gamma camera rather than PET scanner	False positives for benign bone lesions [34] False negatives for osteolytic lesions [33] Poor spatial resolution of traditional planar scintigraphy Requires 2–4 hour wait prior to imaging [45]
^{18}F-NaF	Forms fluoroapatite in bone crystal [46]	Blood flow and bone remodeling	Shorter imaging times and improved accuracy compared with ^{99m}Tc-MDP [44, 45] Highly sensitive for detection of osteolytic, osteoblastic, and mixed lesions	Only detects bone lesions False positives for benign bone lesions High cost

^{18}F-FDG ^{18}F-fluorodeoxyglucose, *^{18}F-NaF* ^{18}F-sodium fluoride, *^{99m}Tc-MDP* technetium ^{99}m-methyl diphosphonate, *PET* positron-emission tomography

Assessment of Residual Tumor After TURBT

Transurethral resection of bladder tumor (TURBT) is a widely used diagnostic and therapeutic procedure in which bladder lesions are biopsied and completely resected to establish a diagnosis of bladder cancer, provide tumor staging information, and begin treatment. In the short-term following TURBT, it can be difficult to determine whether a bladder lesion seen on PET/CT represents true residual tumor or whether it is inflammation related to the procedure. Therefore, many patients require repeat TURBT to evaluate for residual tumor and to ensure a complete resection is performed. Several studies have investigated the utility of PET/CT to assess for residual tumor after TURBT.

A retrospective study evaluated the performance of ^{18}F-fluorodeoxyglucose (FDG) PET/CT in differentiating between residual tumor and postoperative inflammation [1]. They enrolled 79 patients with histologically confirmed bladder cancer who underwent PET/CT within 1 month of initial TURBT, followed by repeat TURBT within 2 weeks to confirm histology of residual masses. Patients underwent both routine whole-body ^{18}F-FDG PET/CT, as well as diuretic delayed PET/CT images after administration of 40 mg oral furosemide. Among 79 patients, 98 bladder lesions were identified, of which 34 (34.7%) were inflammatory and 64 (65.3%) were residual tumors. Using the diuretic delayed PET/CT images, residual tumors had a significantly higher mean standardized uptake value (SUV_{mean}) ($p < 0.001$), maximum standardized uptake value (SUV_{max}) ($p = 0.01$), and thickness ($p < 0.001$) compared to inflammatory lesions. Using a threshold SUV_{mean} of 8.7 and lesion thickness of 12.8 mm, patients were divided into low- ($SUV_{mean} \leq 8.7$ and lesion thickness ≤ 12.8 mm), medium- (either $SUV_{mean} > 8.7$ or lesion thickness > 12.8 mm), and high-risk groups ($SUV_{mean} > 8.7$ and lesion thickness > 12.8 mm). Using this categorization, the rate of residual tumors was 37.5% (17/47) in the low-risk group, 85.4% (26/29) in the moderate-risk group, and 98.3% (21/22) in the high-risk group.

While this study demonstrated that SUV_{mean} and lesion thickness may be useful imaging characteristics on ^{18}F-FDG PET/CT to aid in the differentiation of residual tumor versus inflammatory reaction following TURBT, it remains to be seen whether this could be incorporated into clinical practice. It is possible that patients with low-risk lesions may be able to undergo imaging surveillance rather than a repeat TURBT, but nearly 40% of the lesions categorized as low-risk actually contained residual tumor, and so additional work is needed to improve the accuracy of this risk categorization system.

Bladder Cancer Staging

For patients diagnosed with muscle-invasive bladder cancer (MIBC), accurate evaluation of the primary tumor, lymph node involvement, and metastatic spread is essential for prognostication and appropriate management. Current guidelines from

the National Comprehensive Cancer Network (NCCN) recommend staging imaging with chest CT and abdominal/pelvic CT or MRI [2]. However, staging of MIBC by conventional CT imaging is frequently inaccurate, leading to both under- and over-staging. One retrospective analysis of 276 patients found that CT had only 49% accuracy in predicting pathologic tumor stage and 54% accuracy in predicting lymph node metastases [3]. Recognizing the limitations of conventional imaging modalities, many studies have evaluated the use of PET imaging with conventional and novel radiotracers to improve the staging of bladder cancer.

^{18}F-FDG PET/CT

To date, most studies have focused on the use of ^{18}F-FDG PET/CT for both primary tumor and lymph node staging in bladder cancer, as this is currently the most widely used and readily available radiotracer.

Primary Tumor Staging

Complete staging of a primary bladder tumor depends on an assessment of the depth of tumor invasion into the bladder wall. This is generally accomplished through TURBT, which also provides valuable information regarding the tumor grade and histology. Prior studies have evaluated the role of ^{18}F-FDG PET/CT in bladder tumor staging; however, detailed evaluation of primary bladder tumors is hindered by the urinary excretion of ^{18}F-FDG. Forced diuresis protocols or catheter placement with retrograde bladder filling may improve tumor evaluation, but even using these methods ^{18}F-FDG PET/CT is typically only able to evaluate the presence or absence of tumor in the bladder, not the depth of invasion, thus limiting its utility in staging. A summary of studies evaluating the primary tumor is listed in Table 7.2.

For example, Kibel et al. used a forced diuresis protocol to evaluate the use of ^{18}F-FDG PET/CT for preoperative staging in patients with clinical stage T2-T3 MIBC with no evidence of locoregional or metastatic disease on conventional imaging [4]. Among 41 patients who underwent radical cystectomy (RC) without neoadjuvant chemotherapy (NAC), residual primary tumor was identified pathologically in 34 patients with increased FDG uptake seen in the primary tumor in 28 (sensitivity 82%) of these cases. Similarly, Lodde et al. compared the accuracy of ^{18}F-FDG PET/CT with conventional CT in 44 patients diagnosed with MIBC scheduled to undergo RC without NAC [5]. In these patients, PET/CT had a sensitivity of 85% and specificity of 25% for detecting the primary tumor, compared to 77% sensitivity and 50% specificity of CT.

Additional PET/CT imaging techniques that may improve bladder tumor evaluation are under investigation, including the addition of early dynamic FDG PET in

Table 7.2 Selected studies of PET imaging characteristics for detection of primary bladder tumor

Author	Year	Imaging modality	*n*	Reference standard	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Kibel	2009	^{18}F-FDG PET/CT	41	Pathology	82	NR	NR	NR	NR
Lodde	2010	^{18}F-FDG PET/CT	44	Pathology	85	25	92	14	80
		CT	35		77	50	92	22	74
Rosenkrantz	2017	^{18}F-FDG PET/MRI	22	Pathology or clinical follow-up	89	75	94	60	86
		MRI	22		72	100	100	44	77
Eulitt	2020	^{18}F-FDG PET/MRI	18	Pathology	80	56	70	69	69
		CT	18		91	43	71	75	72
Picchio	2006	^{11}C-choline PET	27	Pathology	96	0	92	0	89
		CT	27		84	50	95	20	85
Golan	2011	^{11}C-choline PET/CT	18	Pathology or clinical follow-up	100	NA	100	NA	100
		^{18}F-FDG PET/CT	18		75	NA	100	0	75
Orevi	2012	^{11}C-choline PET/CT	13	Pathology	92	100	100	50	92
		^{11}C-acetate PET/CT	13		92	100	100	50	92
Brunocilla	2014	^{11}C-choline PET/CT	26	Pathology	69	N/A	100	0	69
		CT	26		92	N/A	100	0	92
Ceci	2015	^{11}C-choline PET/CT	39	Pathology	64	N/A	100	0	64
Vargas	2012	^{11}C-acetate PET/CT	16	Pathology	78	71	78	71	75
		MRI							56
		CT							63
Salminen	2018	^{11}C-acetate PET/CT	15[a]	Pathology	100	69	33	100	73
			5[b]	Pathology					40

^{18}F-FDG ^{18}F-fluorodeoxyglucose, *CT* computed tomography, *MRI* magnetic resonance imaging, *N/A* not applicable, *NPV* negative predictive value, *NR* not reported, *PET* positron-emission tomography, *PPV* positive predictive value, *TURBT* transurethral resection of bladder tumor

[a]Treatment naïve patients who underwent PET/CT prior to initial TURBT

[b]Patients with muscle-invasive bladder cancer who received neoadjuvant chemotherapy. PET/CT performed following chemotherapy and prior to radical cystectomy with lymph node dissection

which serial images of the pelvis are acquired over a period of 10 minutes beginning at the time of radiotracer injection, before the tracer accumulates in urine [6, 7]. This is performed alongside the standard method of whole-body image acquisition beginning 60 minutes after radiotracer injection. Initial studies suggest that early dynamic FDG PET/CT may have a role in predicting tumor grade or depth of invasion [6, 7], but this remains to be validated in larger patient cohorts. Based on the evidence to date, ^{18}F-FDG PET/CT does not provide additional primary tumor staging information beyond that of TURBT with conventional imaging and therefore is not routinely recommended for this use [8].

Lymph Node Staging

In addition to staging the primary tumor, assessment of lymph node metastases is crucial in determining management for patients with bladder cancer. Lymph node staging has traditionally been performed with CT or MRI. Using these modalities, lymph nodes larger than 8 mm or 10 mm are considered suspicious. This size-based criterion leads to both false positives, particularly in the setting of reactive lymph node enlargement following TURBT, and false negatives in the setting of micrometastatic disease. The addition of metabolic information via ^{18}F-FDG PET/CT has been hypothesized to improve the accuracy of lymph node staging compared to conventional imaging; however, results to date have been mixed. A summary of studies evaluating lymph node staging in bladder cancer is listed in Table 7.3.

A meta-analysis evaluated the pooled sensitivity and specificity for the detection of lymph node metastases using CT, MRI, or PET/CT [9]. They found that PET/CT had 56% sensitivity and 92% specificity compared to 60% sensitivity and 91% specificity with MRI and 40% and 92% with CT, respectively. Notably, there was a large range in reported sensitivity and specificity across the included studies, likely owing to considerable heterogeneity regarding study design, patient inclusion criteria, definition of suspicious lymph nodes, experience level of the interpreting radiologist, and use of clinical and/or pathological data to define the reference standard.

Several studies have also evaluated whether there is an added benefit to lymph node assessment using ^{18}F-FDG PET/CT over conventional CT alone. One study included 93 patients with confirmed MIBC or recurrent high-risk non-muscle-invasive bladder cancer (NMIBC) who underwent both ^{18}F-FDG PET/CT and conventional CT imaging prior to cystectomy [10]. Patients who received NAC or who had an inadequate lymphadenectomy (<10 nodes removed) were excluded. Both PET alone and CT alone had a sensitivity of 46% with a specificity of 97% and 98%, respectively. However, the combination of PET and CT together resulted in a sensitivity of 68% and specificity of 95%.

Table 7.3 Selected studies of PET imaging characteristics for evaluation of lymph node metastases

Author	Year	Imaging modality	*n*	Reference standard	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Kibel	2009	^{18}F-FDG PET/CT	42	Pathology	70	94	78	91	88
Swinnen	2009	^{18}F-FDG PET/CT	51	Pathology	46	97	86	84	84
		CT	51		46	92	67	83	80
Lodde	2010	^{18}F-FDG PET/CT	43	Pathology	57	100	100	80	77
		CT	33		33	100	100	77	70
Goodfellow	2014	^{18}F-FDG PET/CT	93	Pathology	68	95	86	87	87
		^{18}F-FDG PET	93		46	97	87	81	82
		CT	93		46	98	93	81	83
Rosenkrantz	2017	^{18}F-FDG PET/MRI	21	Pathology or clinical follow-up	88	100	100	93	95
		MRI	21		38	100	100	72	76
Eulitt	2020	^{18}F-FDG PET/MRI	18	Pathology	0	100	0	83	83
		CT	18		0	93	0	82	78
Picchio	2006	^{11}C-choline PET	27	Pathology	63	100	100	86	89
		CT	27		50	68	40	76	63
Golan	2011	^{11}C-choline PET/CT	18	Pathology or clinical follow-up	NR	NR	79[a]	NR	NR
		^{18}F-FDG PET/CT	18		NR	NR	88[a]	NR	NR
Maurer	2011	^{11}C-choline PET/CT	44	Pathology	58	66	39	81	64
		CT	44		75	56	39	86	61
Orevi[b]	2012	^{11}C-choline PET/CT	13	Pathology	100	NR	50	NR	NR
		^{11}C-acetate PET/CT	13		100	NR	50	NR	NR
Brunocilla	2014	^{11}C-choline PET/CT	26	Pathology	42	84	50	80	73
		CT	26		14	89	33	74	69
Ceci	2015	^{11}C-choline PET/CT	39[c]	Pathology	50	89	67	80	77
		^{11}C-choline PET/CT	20[d]	Pathology	80	93	80	93	90

(continued)

Table 7.3 (continued)

Author	Year	Imaging modality	*n*	Reference standard	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Vargas	2012	^{11}C-acetate PET/CT	16	Pathology	100	71	33	100	75
		MRI	16		50	71	20	91	69
		CT	16		50	79	25	92	75
Salminen	2018	^{11}C-acetate PET/CT	5	Pathology	50	67	50	67	60

Patient-level analyses are reported, except where otherwise noted

^{18}F-FDG ^{18}F-fluorodeoxyglucose, *CT* computed tomography, *MRI* magnetic resonance imaging, *NPV* negative predictive value, *NR* not reported, *PET* positron-emission tomography, *PPV* positive predictive value

[a]Results for detection of any extravesical disease, including lymph nodes and distant metastatic disease

[b]Results for lymph-node based analysis

[c]Patients underwent PET/CT prior to radical cystectomy and lymph node dissection for bladder cancer

[d]Patients with prior radical cystectomy and lymph node dissection for bladder cancer with suspicion for nodal relapse clinically or on conventional imaging (ultrasound, CT, or MRI) who underwent PET/CT followed by lymph node biopsy or salvage lymph node dissection

The second study by Kollberg and colleagues evaluated the added value of ^{18}F-FDG PET/CT compared to CT alone specifically in patients with high-risk MIBC [11]. They enrolled 103 patients fit for cystectomy with high-risk MIBC defined as cT3-4a, cT2 with hydronephrosis, or cT2 with high-risk variant histology. All patients underwent both conventional CT and ^{18}F-FDG PET/CT, which were reviewed at a multidisciplinary board conference to arrive at a definitive treatment plan. PET/CT showed findings suggestive of metastatic disease or additional malignancy that were not found on CT alone in 48 (47%) patients. Importantly, PET/CT findings altered the treatment plan in 28 patients (27%), leading to the cancellation of cystectomy in 16 patients due to findings of disseminated disease and extended neoadjuvant chemotherapy for 12 patients. Additionally, three of the patients had findings on PET/CT that were later determined to be false positives, and evaluation of these ultimately benign findings delayed treatment of MIBC by about 2 months in two of these cases and did not delay cystectomy in the third. Most PET/CT findings were not confirmed pathologically, and therefore, it is unclear if there were additional false positives that led to an inappropriate change in treatment. A multicenter randomized controlled trial is currently ongoing in Canada to further address this question of the clinical value of ^{18}F-FDG PET/CT over CT alone in staging patients with MIBC (NCT02462239).

Given this data, ^{18}F-FDG PET/CT may enhance detection of lymph node and distant metastatic disease compared with conventional imaging alone, although

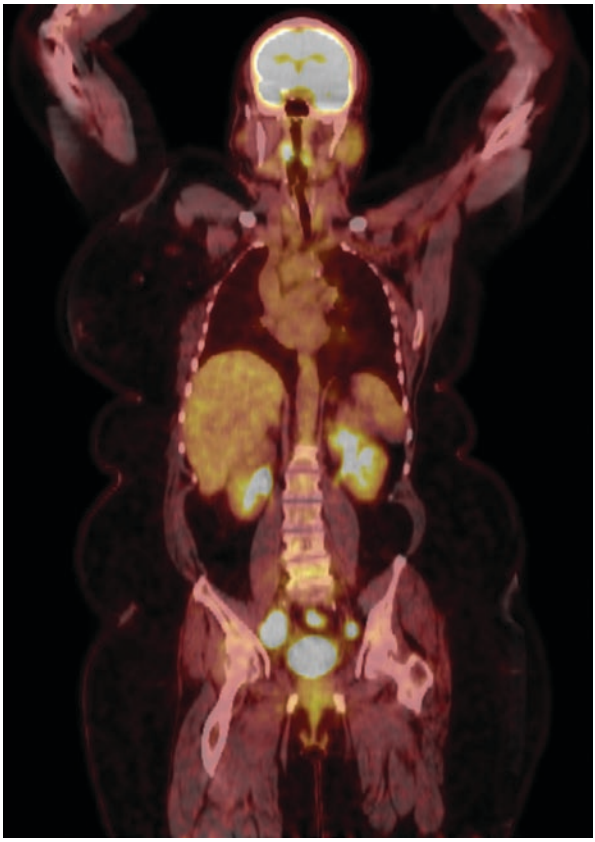

Fig. 7.1 ^{18}F-FDG PET/CT image of a patient with bladder cancer metastatic to bilateral pelvic sidewall lymph nodes. This image also demonstrates the difficulty in the visualization of an underlying bladder mass due to urinary excretion of the radiotracer

there remains limited sensitivity for lymph node metastases. Although PET/CT is less reliant on lymph node size compared to CT or MRI, assessment of lymph nodes <10 mm is still limited. With the high financial cost of PET/CT and incremental added value, appropriate patient selection for PET/CT remains important. PET/CT may be of greater yield in the subset of patients with high-risk disease, including those with indeterminate findings on conventional imaging. PET/CT is likely to add little or no information for patients with NMIBC, and routine use in this setting is not endorsed by guidelines [2, 12, 13]. An ^{18}F-FDG PET/CT image showing intensively avid bilateral pelvic sidewall lymph nodes in patient with an underlying bladder mass that is hard to discern due to renal radiotracer excretion is seen in Fig. 7.1.

^{18}F-FDG PET/MRI

Compared to CT imaging, MRI may provide additional anatomical information, including greater soft tissue detail. PET/MRI has therefore emerged as a novel imaging modality to combine this benefit of MRI with the metabolic information provided by PET and has been evaluated for use in patients with MIBC.

One pilot study compared the sensitivity and specificity of ^{18}F-FDG PET/MRI versus MRI alone for detecting the presence of bladder tumor, pelvic nodal metastases, and non-nodal pelvic metastases in 22 patients with bladder cancer [14]. MRIs were initially reviewed by a radiologist who rated the probability of tumor in each of the three locations on a 1–3 scale (1 = negative, 2 = equivocal, 3 = definite tumor). Subsequently, PET/MRI images were reviewed by a nuclear medicine physician, who adjusted scores based on the combined findings. Using a threshold score of 3, PET/MRI had higher accuracy than MRI alone for the detection of bladder tumor (86% vs 77%), metastatic pelvic lymph nodes (95% vs 76%), and non-nodal pelvic metastases (100% vs 91%). Additionally, the PET information changed the level of suspicion for bladder tumor in 36% of patients, for pelvic lymph node metastases in 52% of patients, and for non-nodal pelvic metastases in 9% of patients.

A second pilot study evaluated ^{18}F-FDG PET/MRI compared with conventional CT imaging for staging of MIBC, with surgical pathology results as the reference standard [15]. This study included 18 patients, the majority of whom (72%) received neoadjuvant chemotherapy prior to PET/MRI. PET/MRI had 80% sensitivity, 56% specificity, and 69% accuracy for evaluation of the primary tumor and 0% sensitivity, 100% specificity, and 83% accuracy for evaluation of lymph node involvement. However, results were limited by the small number of patients with pathologic lymph node involvement at cystectomy (3/18; 17%). CT imaging performed similarly to PET/MRI, with 91% sensitivity, 43% specificity, and 72% accuracy for primary tumor and 0% sensitivity, 93% specificity, and 78% accuracy for lymph node involvement.

As pilot studies, both included a small number of patients, some of whom had received prior therapy that could have influenced imaging interpretation. PET/MRI technology is also relatively new and has novel technical challenges, including combining free-breathing PET data with breath-holding MRI data. As experience with PET/MRI increases, so too may its diagnostic utility. As seen with ^{18}F-FDG PET/CT, PET/MRI may be useful in staging of MIBC, particularly to help characterize lesions that are indeterminate on conventional imaging, but further evaluation is needed.

^{11}C-Choline PET/CT

Although ^{18}F-FDG is the most widely used radiotracer for PET imaging in oncology, its urinary excretion can interfere with the imaging of tumors in the urinary tract. In contrast, ^{11}C-choline has negligible urinary excretion, which has spurred its evaluation for use in staging of bladder cancer.

An early study from Picchio and colleagues included 27 patients with bladder cancer who underwent cystectomy and pelvic lymph node dissection (PLND) without NAC [16]. Patients received ^{11}C-choline PET, conventional CT, and bone scan prior to surgery, which were compared to histopathologic results. CT correctly identified 21 and ^{11}C-choline PET correctly identified 24 of 25 residual bladder tumors (sensitivity 84% and 96%, respectively), while CT identified 4 and ^{11}C-choline PET identified 5 of 8 patients with lymph node metastases (sensitivity 50% and 62.5%, respectively). CT had an overall accuracy of 63% for categorizing lymph node involvement, while PET was 88.9% accurate ($p < 0.01$). This decreased accuracy of CT was primarily related to false positives, with 6 false-positive nodes identified on CT and 0 false-positive nodes on PET, suggesting that ^{11}C-choline PET may be particularly useful in characterizing indeterminate or borderline nodes seen on CT.

Additional studies have compared ^{11}C-choline PET/CT with conventional CT for nodal staging of bladder cancer. In contrast to the earlier study by Picchio, these studies used combined PET/CT imaging to provide co-registered anatomic and functional images. Maurer et al. enrolled 44 patients with localized bladder cancer scheduled for cystectomy and PLND without NAC [17]. An analysis was performed based on 14 pre-defined anatomic lymph node fields, where the presence or absence of lymph node metastases for each field was rated based on imaging and compared to pathologic results of lymph nodes removed from that field. Both PET/CT and CT alone had low sensitivity (27.5% and 38.7%, respectively), high specificity (94.7% and 92.2%, respectively), and nearly identical accuracy (90.9% and 89.5%, respectively) in this analysis. In a patient-level analysis, PET/CT had lower sensitivity compared to CT alone (58.3% vs 75%) but higher specificity (65.6% vs 56.3%) and accuracy (63.6% vs 61.4%). A similar study of nodal metastases in 26 patients by Brunocilla et al. found somewhat dissimilar results, with PET/CT demonstrating a higher sensitivity (42% vs 14.3%) and accuracy (73% vs 69%) but a lower specificity (84% vs 89.5%) compared to CT alone in a patient-level analysis [18]. In a lymph node-based analysis, both PET/CT and CT performed poorly with low sensitivity (10.5% vs 2.0%), modest specificity (64% vs 63%), and low accuracy (31.7% vs 27.7%). The role of ^{11}C-choline PET/CT therefore remains investigational.

^{11}C-Acetate PET

A third radiotracer that has been investigated for use in bladder cancer staging is ^{11}C-acetate PET/CT. The potential utility of this tracer was described in a study comparing ^{11}C-acetate PET/CT with ^{11}C-choline PET/CET in 13 patients with bladder cancer prior to cystectomy [19]. Both tracers demonstrated true-positive uptake in the bladder in 11 patients, true-negative uptake in 1 patient, and false-negative uptake in 1 patient with remaining carcinoma in situ. Similarly, both tracers had increased uptake in 10 total lymph nodes, 5 of which were true positive and 5 were false positive, with all other removed lymph nodes confirmed as true negatives.

Vargas et al. compared ^{11}C-acetate PET/CT with both MRI and conventional CT for the detection of primary tumor and nodal metastases in 16 patients with bladder

cancer undergoing cystectomy and PLND [20]. ^{11}C-acetate PET/CT had a sensitivity of 78% and specificity of 71% for the detection of residual primary bladder tumor. MRI determined the correct T stage in 56% of patients, overstaged 38%, and understaged 6%, while CT correctly staged 63%, overstaged 31%, and understaged 6%. Only two patients had pathologically confirmed nodal metastases, which were both correctly identified by PET/CT (sensitivity 100%), while MRI and CT each only identified one (sensitivity 50%). Specificity was similar across modalities with PET/CT and MRI demonstrating a specificity of 71% and CT demonstrating a specificity of 79%.

Salminen et al. evaluated the performance of ^{11}C-acetate PET/MRI for staging of bladder cancer in 15 patients prior to initial TURBT and for the evaluation of response to NAC prior to cystectomy and PLND in 5 patients [21]. ^{11}C-acetate PET/MRI had 100% sensitivity, 69% specificity, and 73% accuracy in detecting the presence of muscle-invasive disease on initial TURBT. Among the five patients who received NAC, PET/MRI correctly staged two as T0, understaged one, and overstaged two. At the time of surgery, lymph nodes were removed and evaluated from 10 predetermined regions. The sensitivity, specificity, and accuracy of PET/MRI for the detection of nodal metastases at the level of the predetermined nodal regions were 20%, 96%, and 88%, respectively. In a patient-level analysis, the sensitivity, specificity, and accuracy were 50%, 67%, and 60%, respectively. Notably, one patient who underwent cystectomy had bilateral hip prostheses, which created significant image distortion. This patient was staged as T0 N0 on PET/MRI yet had T3 disease with nine positive lymph nodes at the time of surgery, highlighting a limitation of this imaging modality.

A systematic review and meta-analysis included ten studies that evaluated the use of ^{11}C-choline and ^{11}C-acetate PET/CT for preoperativei lymph node staging in patients with bladder cancer [22]. These studies included a total of 282 patients with a pooled sensitivity of 66% (95% CI 54–75%) and a pooled specificity of 89% (95% CI 76–95%); however, there was significant between-study heterogeneity for specificity. Prospectively designed studies had a significantly lower specificity compared to retrospective studies (74% vs 95%, $p < 0.01$; $n = 5$ for both), while both study designs resulted in similar sensitivity (68% vs. 64%, $p = 0.74$). The results of this meta-analysis are similar to those reported in a meta-analysis of ^{18}F-FDG PET/CT for nodal staging, which had a pooled sensitivity of 57% and specificity of 92% [23].

Additionally, one study directly compared ^{11}C-choline PET/CT with ^{18}F-FDG PET/CT in 18 patients with bladder cancer [24]. This study included patients prior to primary treatment and in follow-up after cystectomy, which necessitated the use of both histological results and follow-up imaging as the standard of reference. ^{11}C-choline PET/CT had a positive predictive value (PPV) of 100% for the detection of primary bladder tumor and 79% for the detection of extravesical lesions, while ^{18}F-FDG PET/CT had a sensitivity PPV of 100% for primary bladder tumor and 88% for extravesical lesions. ^{11}C-choline and ^{18}F-FDG uptake was discordant for three bladder lesions and eight extravesical lesions. All three discordant bladder lesions were found to be true positives on ^{11}C-Choline PET/CT and false negatives on ^{18}F-FDG PET/CT. Of the eight discordant extravesical lesions, ^{11}C-choline PET/

CT correctly characterized two and ^{18}F-FDG PET/CT correctly characterized six. Although limited by a small and heterogeneous patient sample, this study does not suggest a significant advantage to ^{11}C-choline over ^{18}F-FDG, and in fact, ^{18}F-FDG had higher sensitivity and specificity for extravesical lesions.

Overall, the current evidence to support the use of ^{11}C-choline or ^{11}C-acetate PET/CT for staging of bladder cancer prior to cystectomy is limited. The potential benefit of either tracer in terms of staging accuracy over conventional imaging (or over ^{18}F-FDG PET/CT) has not been consistent across studies, and research thus far has included few patients. Furthermore, the half-life of ^{11}C-choline and ^{11}C-acetate is only around 20 minutes, which limits their use to facilities with an on-site cyclotron. Based on these limitations, the use of either tracer for preoperative staging of bladder cancer is not recommended by major guidelines [2, 25].

Response to Neoadjuvant Chemotherapy

Neoadjuvant chemotherapy prior to radical cystectomy is recommended for patients with MIBC and pathologic downstaging at the time of surgery is associated with increased overall survival [26]. Chemotherapy response assessment for nodal metastases with conventional CT is hampered by multiple factors, including limited detection of lymph node metastases <10 mm, inconsistent correlation between a reduction in lymph node size with a histological response, and difficulty with identifying viable tumor in a residual mass. Based on these limitations, the use of ^{18}F-FDG PET/CT in determining response to NAC has been evaluated in several studies [27–30].

The first study included 19 patients with MIBC and clinically node-positive disease on the initial staging imaging [29]. All patients received neoadjuvant platinum-based chemotherapy, followed by repeat ^{18}F-FDG PET/CT and conventional CT at least 2 weeks after completing NAC. All patients then underwent PLND, with 14 achieving pN0 and 16 achieving any nodal downstaging. PET/CT correctly distinguished responders from nonresponders in 18 cases (94.7%), while CT alone correctly categorized 15 patients (78.9%). Additionally, PET/CT correctly distinguished complete responders from those with residual disease in 13 cases (68.4%), while CT alone correctly distinguished 12 cases (63.2%). This corresponded to a sensitivity and specificity of 71% and 60% for PET/CT detection of complete responders, compared with 64% and 60% for CT alone. Although the sample size was small, PET/CT appeared to perform very similarly to CT in regard to the detection of nodal complete responders. Similarly, a study of 37 patients with cT1-4 cN1-3 bladder cancer found that ^{18}F-FDG PET/CT correctly identified 16 of 24 patients with complete nodal response, with a corresponding sensitivity of 67% and specificity of 46% [27]. In many cases of persistent nodal FDG activity, the pathology revealed a complete response, resulting in a PPV of 70% and an NPV of 43%.

A study by Soubra et al. also evaluated the accuracy of ^{18}F-FDG PET/CT in detecting complete tumor response following NAC [30]. A total of 37 patients with MIBC underwent PET/CT before and after NAC. Using a 100% change in SUV_{max} as a threshold for determining complete response, PET/CT correctly identified 6 of 8 patients (sensitivity 75%) with pathologic complete response (pCR) and 26 of 29 patients (specificity 89.7%) without pCR. In contrast to the prior two studies, this study did not evaluate nodal response to NAC, which may partly explain the higher sensitivity and specificity reported here.

In addition to determining response following completion of NAC, ^{18}F-FDG PET/CT has also been evaluated for use in response prediction during NAC [28]. In theory, this strategy could lead to early identification of patients with chemotherapy-insensitive tumors, preventing overtreatment with additional chemotherapy and preventing additional delay in cystectomy. Patients with a partial response could be selected to undergo additional chemotherapy in hopes of achieving a complete response.

In the previously described study by Soubra et al., 20 patients completed an additional PET/CT after 2 cycles of chemotherapy [30]. Using a 50% reduction in SUV_{max} as a threshold to identify chemosensitive tumors with subsequent downstaging to less than pT2, PET/CT had a sensitivity of 57% and specificity of 92%. Kollberg et al. also assessed NAC response in patients with cT1-4a disease and initial staging ^{18}F-FDG PET/CT findings indicating node-positive disease, oligometastatic disease in the retroperitoneum, or a single bone metastasis [28]. Patients received three cycles of NAC followed by a second PET/CT, with three patients demonstrating metastatic disease progression during chemotherapy on this follow-up imaging. Of 43 patients with initially node-positive disease, mid-NAC PET/CT correctly identified 37/37 patients with nodal response but only 1/6 patients without nodal response, with a corresponding sensitivity of 100%, specificity 17%, and accuracy 88%. However, patients with either a complete or partial nodal response were considered "responders" in this analysis, unlike prior studies which separately evaluated patients with a complete response.

These studies highlight several important considerations with the use of ^{18}F-FDG PET/CT for NAC response assessment. First, the optimal timing of PET/CT in relation to NAC is unclear. PET/CT performed either mid-chemotherapy or shortly after chemotherapy completion may overestimate true pathologic response, but a prolonged time interval could create unnecessary treatment delays. Second, findings of clinically node-positive disease on the initial staging PET/CT are typically not confirmed histologically prior to the initiation of chemotherapy. Therefore, it is possible that initial false positives, such as reactive lymph nodes in the setting of recent TURBT, could influence ultimate outcomes. However, the pooled specificity of FDG PET/CT for lymph node metastases was 92–95% in meta-analyses [23, 31], and so a significant number of false positives are less likely. Finally, based on the current evidence, PET/CT does not appear to be sufficiently accurate to guide changes in treatment plans during or after NAC, particularly for patients with suspected nodal metastases.

Detection of Bone Metastases

The bone is a common site of spread for bladder cancer, with bone metastases reported in over 40% of patients with metastatic disease [32]. Early detection of bone involvement is important, as bone metastases can cause pain and lead to complications including fracture and spinal cord compression. Plain film radiographs can visualize osteolytic lesions, only after 50–70% of the bone is demineralized, and are therefore of limited utility, particularly for the detection of asymptomatic metastases [33]. Conventional CT imaging can detect cortical involvement of bone metastases, while MRI can evaluate the presence of intramedullary metastatic disease prior to cortical destruction, resulting in earlier detection with improved sensitivity for these modalities compared to plain films [33].

In addition to the structural information provided by plain films, CT, and MRI, newer nuclear imaging techniques now allow for an assessment of bone activity. The most widely used radiotracer for bone scintigraphy for the detection of bone metastases is technetium ^{99}m-methyl diphosphonate (^{99m}Tc-MDP), which accumulates at sites of active bone production. The spatial resolution of traditional planar scintigraphy is poor, and determining the precise location of a lesion can be difficult, although single-photon emission computerized tomography (SPECT)/CT, which acquires cross-sectional imaging, can be used for improved localization. Additionally, ^{99m}Tc-MDP is only able to assess the presence of an osteoblastic process, resulting in possible false-negative results for osteolytic lesions. In comparison, ^{18}F-sodium fluoride (^{18}F-NaF) PET/CT provides greater spatial resolution and accumulates in both osteoblastic and osteolytic lesions. These characteristics make it an attractive candidate for the evaluation of bone metastases in bladder cancer in particular, as they frequently have a mixed osteolytic and osteoblastic phenotype.

One study compared ^{18}F-NaF PET/CT with ^{99}mTc-MDP planar bone scan and SPECT/CT for detecting bone metastases in 48 patients with newly diagnosed locoregional or metastatic bladder cancer [34]. All patients underwent planar bone scan with SPECT/CT performed only for suspicious areas on planar imaging followed by ^{18}F-NaF PET/CT within 2 days. Bone metastases were confirmed based on imaging follow-up or definitive biopsy. ^{18}F-NaF PET/CT accurately identified bone involvement in 17/17 patients (sensitivity 100%) and excluded involvement in 27/31 patients (87.1% specificity), for an overall accuracy of 91.7%. Both SPECT/CT and planar bone scan had decreased sensitivity (88.2% and 82.4%, respectively), specificity (74.2% and 64.5%, respectively), and accuracy (79.2% and 70.8%, respectively) compared to PET/CT.

^{18}F-FDG PET/CT has also been evaluated for use in the evaluation of distant metastatic disease in bladder cancer with generally high sensitivity and specificity, but there are limited data regarding the evaluation of bone metastases specifically [8]. Lodde et al. compared ^{99m}Tc-MDP planar bone scan with ^{18}F-FDG PET/CT in 36 patients and found that both detected bone metastases in the same three patients [5]. In one of these cases, additional pelvic and vertebral bone metastases were

detected only on FDG PET/CT. Apolo et al. performed an organ-specific analysis of ^{18}F-FDG PET/CT for the detection of metastatic disease in 47 patients [35]. Bone metastases were identified at 22 sites with a sensitivity of 93% and specificity of 100%.

Both ^{18}F-NaF and ^{18}F-FDG PET/CT appear to have high sensitivity and specificity for bone metastases; however, they both also have a high financial cost. While ^{18}F-NaF PET/CT can accurately evaluate bone disease, ^{18}F-FDG PET/CT has the advantage of using metabolic activity to also detect extraosseous metastatic disease and is therefore generally favored. NCCN guidelines recommend that symptomatic patients, high-risk patients, or patients with laboratory indicators of bone metastasis may undergo imaging with MRI, ^{18}F-FDG PET/CT, or bone scan, and comment that FDG PET/CT may be considered particularly when extraosseous metastatic disease is suspected or proven [2].

Radiotracers and Immunotherapy

Immune checkpoint blockade with inhibitors of programmed cell death protein 1 (PD-1) or its ligand (PD-L1) has emerged as a standard treatment modality for advanced bladder cancer, with five agents now approved by the Food and Drug Administration in this setting [36]. Despite encouraging results with some patients achieving a durable response, overall response rates with these agents remain around 20–30%. Predicting which patients will respond to anti-PD-(L)1 therapy using currently available biomarkers, such as tumor PD-L1 staining, remains imperfect. This results in a significant percentage of patients receiving these agents who do not derive clinical benefit but who are exposed to the potential for serious immune-related adverse events. Therefore, it is important to identify responders as early as possible to avoid unnecessary treatment or toxicity and to allow earlier treatment with other effective agents.

In contrast to tissue-based biomarkers, molecular imaging modalities are noninvasive, can assess all tumor sites simultaneously, and can be more easily repeated over time to monitor disease response. With these advantages, several novel radiotracers have been developed with the goal of improving the prediction of response to immunotherapy. The feasibility of zirconium-89-labeled atezolizumab (^{89}Zr-atezolizumab) PET imaging was tested in 22 patients with locally advanced or metastatic bladder cancer, non-small cell lung cancer, or triple-negative breast cancer, who were treated with atezolizumab [37]. On imaging prior to treatment, tumor ^{89}Zr-atezolizumab uptake was generally high but with significant within-patient and intra-tumor heterogeneity observed. Overall, 3 patients (14%) had complete response, 4 patients (18%) had partial response, and 11 patients (50%) had stable disease as the best response, with baseline geometric mean SUV_{max} increasing with increasing tumor response category ($p_{trend} < 0.001$). Patients with a geometric mean SUV_{max} below the median were significantly more likely to experience disease

progression or death compared with those whose uptake was above the median (progression-free survival (PFS) HR 11.7, 95% CI 3.3–62.7, $p = 0.000028$; overall survival (OS) HR 6.3, 95% CI 1.8–33.4, $p = 0.0027$). Notably, increased PD-L1 immunohistochemical expression did not demonstrate a significant relationship with tumor response, PFS, or OS.

Although this was a small pilot study, these results are encouraging and suggest that ^{89}Zr-atezolizumab PET may be a useful predictor of response to atezolizumab treatment. Further studies are needed to evaluate ^{89}Zr-atezolizumab PET in a larger patient population and to also explore whether this radiotracer could be used to predict response to other PD-(L)1 inhibitors. Additional novel radiotracers that may predict response to immune checkpoint inhibitors are in development, including an ^{89}zirconium-labeled minibody against CD8+ T cells [38] and a ^{68}gallium-labeled peptide that binds to granzyme B [39, 40].

Investigational Radiotracers

Multiple radiotracers with demonstrated utility in other clinical scenarios are currently under investigation for use in patients with bladder cancer, including ^{18}F-fluciclovine and ^{15}O-H_2O. ^{18}F-fluciclovine is a synthetic amino acid and is transported across cell membranes by amino acid transporters that are upregulated in some cancer types. ^{18}F-fluciclovine also has minimal activity in excreted urine, which may allow for enhanced bladder and pelvic imaging compared to ^{18}F-FDG. This tracer is currently approved by the Food and Drug Administration for PET/CT evaluation of suspected prostate cancer recurrence in patients with an elevated prostate-specific antigen following prior cancer treatment. A phase I clinical trial is ongoing to evaluate the use of ^{18}F-fluciclovine PET/CT for staging of MIBC compared to conventional CT or MRI (NCT04018053).

^{15}O-H_2O is a metabolically inert radiotracer that can pass freely across cellular membranes. Its clearance depends entirely on the rate of blood flow, which has led to its application as a noninvasive means of measuring perfusion. Research applications have primarily focused on the quantification of myocardial and cerebral blood flow; however, this same principle has also been applied to the measurement of tumor blood flow across different cancer types [41]. It is hypothesized that changes in tumor blood flow or blood volume may accompany or even precede changes in tumor size and, therefore, these may represent valuable markers of clinical response to antineoplastic therapies. The first clinical trial employing ^{15}O-H_2O PET/MRI in patients with bladder cancer is now open to accrual and aims to evaluate whether changes in tumor blood flow before and after neoadjuvant chemotherapy can successfully identify patients with a complete pathologic response to chemotherapy at cystectomy (NCT04321707). If this method is effective, it could potentially be used to select patients appropriate for bladder sparing treatment, which would be an important advance in the management of MIBC.

Imaging Guidelines

Non-muscle-Invasive Bladder Cancer

Guidelines from both the NCCN and the American College of Radiology (ACR) recommend against the use of ^{18}F-FDG PET/CT for staging or surveillance of patients with NMIBC, based on the low risk of metastatic disease and the impaired evaluation of bladder tumors by urinary FDG excretion [2, 42].

Muscle-Invasive and Metastatic Bladder Cancer

Current NCCN and ACR guidelines suggest that ^{18}F-FDG PET/CT may be appropriate for pre-treatment staging of MIBC [2, 25]. NCCN guidelines state that FDG PET/CT may be useful in selected patients with ≥cT2 disease and may change management in patients with ≥cT3 disease (category 2B). Similarly, ACR guidelines state that FDG PET/CT may improve sensitivity for detecting nodal and distant metastatic disease and note that there is increasing evidence that FDG-PET/CT alters patient management compared with other staging tests. For patients who are symptomatic, are high-risk, or have laboratory indicators suggestive of bone metastases, NCCN guidelines suggest that they may be imaged with MRI, FDG PET/CT, or bone scan and that FDG PET/CT may also be considered in patients where extraosseous metastatic disease is suspected or previously documented.

Both NCCN and ACR guidelines suggest that ^{18}F-FDG PET/CT may be appropriate for post-treatment surveillance of MIBC. In this setting, the NCCN states that FDG-PET/CT may be performed if not previously done or for high-risk patients with suspected metastatic disease. ACR guidelines note that FDG-PET/CT may be used to resolve equivocal findings identified on other imaging tests.

In contrast, the 2020 European Association of Urology (EAU) guidelines state that ^{18}F-FDG PET/CT may have clinical utility for staging metastatic bladder cancer but that additional trial results are awaited before a formal recommendation can be made [43]. The use of any radiotracer other than ^{18}F-FDG is not currently endorsed by any of the major guidelines. ACR guidelines state that there is increasing interest in ^{11}C-choline PET/CT for staging and surveillance of MIBC; however, this remains experimental.

Conclusions

The use of molecular imaging modalities in combination with established and novel radiotracers remains an area of active investigation for patients with bladder cancer. Although ^{18}F-FDG PET/CT is now widely employed in oncology, its use in bladder

cancer has been more limited due to urinary excretion resulting in impaired visualization of the bladder and local lymph nodes. Current evidence suggests that ^{18}F-FDG PET/CT is likely of limited utility for primary tumor staging; however, it may be useful for lymph node staging or assessment of distant metastatic disease, particularly in patients with indeterminate findings on conventional imaging. ^{11}C-choline and ^{11}C-acetate both overcome the issue of urinary excretion, but their short half-life necessitates an onsite cyclotron. Use of these radiotracers in bladder cancer staging remains investigational, with preliminary data suggesting similar sensitivity and specificity for lymph node staging as is seen with ^{18}F-FDG PET/CT.

Ongoing studies aim to evaluate whether PET imaging can predict or accurately assess treatment response. If successful, this approach could have a significant impact on bladder cancer management, potentially avoiding unnecessary or ineffective treatment and thereby improving patient outcomes. However, studies to date have included small numbers of patients, and additional investigation is needed to evaluate the utility of each of these radiotracers and the cost-effectiveness of molecular imaging techniques over conventional imaging modalities.

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Chapter 8
Optical Techniques for Bladder Cancer Detection: The Role of Cystoscopy and Enhanced Cystoscopy

Keiko Cooley, Vignesh T. Packiam, and Ryan P. Werntz

Standard White Light Cystoscopy

The basis of modern cystoscopy was created in 1806 [12]. Following several key modifications, contemporary rigid cystoscope sets consist of the following components: sheath, obturator, optical lens, and bridge [12]. Optical lenses are responsible for the transmission of images and have angled tips that range from 0 to 120° [12]. For visualization of the urethra, a 0- or 12- degree lens is optimal [12]. For surveillance or intervention, a 25- or 30- degree lens should be used to facilitate visualization of the majority of the bladder [12]. Visualizing the bladder neck, dome, and inferolateral and anterior walls may require a 70- or, less frequently, a 120-degree lens [12]. In the outpatient setting, when flexible cystoscopy is not available, the utilization of smaller, 15- or 17- French (Fr), sheaths may be used to increase patient comfort [12].

In most countries, flexible cystoscopes have supplanted the use of rigid cystoscopes in the outpatient setting [13]. They provide an improved patient experience secondary to a smaller size, 16- or 17- Fr, easier navigation through the urethra, and improved visualization of the bladder mucosa owing to tip deflection ranging between 120- and 210- degrees [12]. There are two types of flexible cystoscopes: fiber-optic and digital [12]. High-definition digital cystoscopes have significantly higher resolution and depth of field and provide a larger image when compared to standard-definition digital scopes and fiber-optic cystoscopes [12]. Conversely, fiber-optic scopes provide better illumination [12]. Pre-procedural preparation for

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cystoscopy includes sterile preparation of the genitalia with a water-based iodophor-containing product [12]. For rigid cystoscopy, a lubricating gel is placed on the end of the sheath [12], and for flexible cystoscopy, an anesthetizing lubricating gel is injected into the urethra and allowed to dwell for at least 10 minutes [18].

Techniques to Reduce Pain

Outpatient cystoscopic evaluation is the cornerstone of bladder cancer surveillance. Due to the high rate of recurrence, there is a need for regularly scheduled surveillance for non-muscle-invasive bladder cancer, as well as those treated with trimodality, which entails multiple cystoscopic evaluations. Although the procedure is invasive and performed without general anesthesia, there are some interventions to reduce procedural pain. The current standard of care is the use of intraurethral anesthetizing lubricating gels with the best results achieved with dwell times of at least 10 minutes [18]. Although this gel improves the tolerability of the procedure, the Bladder Cancer Advocacy Network has deemed investigation into further techniques to reduce pain a research priority [18].

Recently, adjuncts to anesthetizing lubricating gel have been explored. One technique that leverages an anatomical reflex is instructing male patients to urinate during the passage of the flexible cystoscopy [21]. This prompts relaxation of the external urethral sphincter and has been shown to significantly reduce pain [21]. An alternative technique to distend the membranous urethra is achieved through "bag squeeze," providing a statistically significant reduction in pain [2]. Utilization of distraction techniques has also demonstrated notable results. In a study evaluating real-time visualization, listening to music, and a combination of both, the combination group had the best experience via survey results [15]. Moreover, patients in the combination group demonstrated the smallest change between preoperative and postoperative pulse and systolic blood pressure, reported the lowest postoperative pain, and had the most willingness to repeat the procedure [15]. Regarding rigid cystoscopy, particularly in countries or settings where flexible cystoscopy is not available, Tezcan et al. demonstrated a statistically significant decrease in pain in hypnotized patients undergoing rigid cystoscopy in the outpatient setting [13].

Use of Antibiotics

As antibiotic stewardship is recognized as a priority and the development of multidrug-resistant organisms is increasing, all urologists should be familiar with the AUA's best practice statements regarding antimicrobial prophylaxis for urologic procedures. Data demonstrates a low risk of infection associated with cystoscopy in a healthy, asymptomatic patient with normal genitourinary anatomy [20]. Conversely, there are patients that require antibiotic prophylaxis (AP). If AP is

required, it is most appropriate to target gram-negative rods and enterococci [16]. The first-line antimicrobial choices would be trimethoprim-sulfamethoxazole or amoxicillin/clavulanate [20]. Appropriate alternatives are first- or second-generation cephalosporins or an aminoglycoside with or without ampicillin [20]. The following patients would be appropriate for AP: pregnant women with asymptomatic bacteriuria, anatomic anomalies of the urinary tract that impact forward flow of urine, immunosuppressed/immunodeficient, recent recipients of systemic chemotherapy, externalized catheters, and comorbidities found on the modified frailty index [16].

Enhanced Cystoscopic Modalities

Despite white light cystoscopy (WLC) being the standard of care, enhanced cystoscopic visualization modalities have been explored and studied in an attempt to detect urothelial bladder lesions with higher sensitivity than WLC. To date, two modalities, narrow-band imaging (NBI) and blue light cystoscopy (BLC), have demonstrated clinical value.

Narrow-Band Imaging

NBI is a type of enhanced cystoscopy that improves the visualization of urothelial lesions by emitting and filtering specific wavelengths of the visible light spectrum [4] which highlights the prominent vasculature associated with urothelial malignancies [9]. NBI does not require any additional intravesical instillation, and instead exploits hemoglobin's absorption of light resulting in increased prominence of the vasculature [19]. Although there is no financial burden of intravesical instillation, there is a need to purchase a proprietary system from Olympus™, ranging from $60,000 to 90,000 USD [9]. Within the light source of the NBI system, there are an NBI filter, Xenon lamp, and red-green-blue rotary filter [8]. In the superficial layers of the bladder mucosa, the 415 nm wavelength, visualized as blue, penetrates and reflects the vasculature as brown, secondary to its assignment to the green channel on the rotary filter [8]. The deeper layers are penetrated by the 540 nm wavelength, visualized as green, and are assigned to the red channel of the rotary filter, reflecting the vasculature as cyan [8, 9]. The prominence of vasculature created through the filtration and reflection of light by the NBI system improves the visualization of over-vascularized tissue that is suspicious for malignancy [4].

Comparative studies between NBI and WLC have demonstrated NBI's superiority in the detection of multifocal papillary lesions as well as carcinoma in situ (CIS). Studies have demonstrated that NBI cystoscopy facilitates the detection of 18% more bladder tumors than would be seen with WLC alone [4, 17]. Assessing NBI's

utility in patients with abnormal urine cytology, it was reported that NBI-guided biopsies were able to diagnose non-muscle-invasive bladder cancer in 42% of patients on the first evaluation [4]. When investigating sensitivities, NBI has demonstrated a sensitivity of 95% compared to WLC's sensitivity of 81% [4, 17]. With respect to specificity, WLC is superior to NBI (79% versus 73%, 95% CI (0.69–0.95)) [17]. Most importantly, when evaluating the ability of NBI-assisted TURBT to decrease tumor recurrence rates, studies report a 53% reduction in recurrence at 3 months [17], 10–19% reduction at 12 months [4, 17], and 22% at 24 months [19]. While some data suggests potential reduction in in recurrence rates with the use of NBI, there is no level 1 evidence demonstrating improvement in cancer-specific survival [4].

Blue Light Cystoscopy with TURBT

BLC, also known as photodynamic diagnosis (PDD) and fluorescence cystoscopy, is currently the most widely accepted modality of enhanced) cystoscopy, with greater than 300 studies reporting its efficacy [6, 19]. Similar to NBI, BLC utilizes manipulation of the visible light spectrum to accentuate the demarcation of suspicious urothelium to improve the thoroughness of tumor detection and resection. Early studies established the safety and mechanism of 5-aminolevulinic acid (5-ALA), demonstrating that intravesical instillation resulted in preferential uptake in neoplastic cells without systemic absorption [17, 19]. 5-ALA, however, was found to be a less-than-ideal compound for clinical practice. Compared to its ester, hexaminolevulinate (HAL) chloride, 5-ALA is less lipid soluble at physiological pH and the induced fluorescence of urothelium dissipates rapidly [19]. Presently, only HAL is approved for use in the United States and Europe for the detection of bladder malignancies [5]. One to three hours prior to cystoscopy, HAL is instilled into the bladder [5] and is preferentially concentrated in rapidly dividing cells [6]. In those cells, HAL is converted to photoactive porphyrins (protoporphyrin IX) that emit visible red light, at 600–740 nm, when viewed under blue light, 375–480 nm [4, 6, 7, 17, 19]. The contrast of the fluorescent red lesions juxtaposed on the blue bladder mucosa facilitates a more complete detection and subsequent resection of lesions and has proven to be impactful on tumor recurrence and progression.

Compared to WLC, BLC has demonstrated its superiority in the detection of multifocal lesions and CIS. The results from a prospective multicenter registry reported that adding the use of BLC to WLC increased tumor detection rates for papillary and CIS lesions by 12% and 43%, respectively [6]. These rates have remained consistent with other data reporting an average 14% increase of papillary lesions detected via BLC and a 41% increase of CIS detected [4]. Additional findings from the prospective multicenter registry reported that 25% of patients who were found to be negative for malignancy via WLC were found to have additional tumors under BLC [6]. Further, 6% of patients with multifocal disease were

upgraded to a higher AUA risk stratification category [6]. Additionally, BLC has demonstrated its value in the evaluation of patients with negative WLC but positive urine cytology. In this particular scenario, 83% of lesions were found utilizing BLC alone [4]. The large body of data reporting BLC demonstrates a sensitivity of 76–97% and specificity of 61–90% [4, 7, 19]. The variability in ranges for sensitivity and specificity could be secondary to lesion and/or operator factors [4]. Despite the variance in specificity, evidence has demonstrated that the use of BLC decreases bladder cancer recurrence rates at 12 months when compared to WLC, by approximately 35% and 45%, respectively [4]. Long-term follow-up demonstrated the durability of these findings showing persistent decreased recurrence risk when compared to WLC, by approximately 39% and 53%, respectively [4]. Moreover, there is data that suggests BLC reduces the risk of cancer progression [17, 19]. This is likely secondary to improved visualization of tumors, including CIS, facilitating a more complete resection [17, 19]. Despite the reported decrease in recurrences and increased sensitivity in comparison to WLC, there is no current data that clearly demonstrates improvements in cancer-specific or overall mortality [4].

Flexible Blue Light Cystoscopy

In a recent study by Daneshmand et al., the clinical utility of BLC with HAL was broadened to the outpatient surveillance setting with flexible cystoscopy [7]. The primary efficacy end point for this study was the detection of malignancy by blue light flexible cystoscopy (BLFC) missed by white light flexible cystoscopy (WLFC), and the primary safety end point evaluated the proportion of adverse events following the procedure [7]. Results of the study demonstrated that 46% of the malignancies found were discovered solely by BLFC [7]. In evaluating the false positivity rates, BLFC) and WLFC were equal at 9.1% [7]. Regarding safety, the reported adverse events including bladder pain, spasms, hematuria, and) dysuria could not be definitively associated with the instillation of HAL [4,6,]. Additional data has shown that repeat instillation of HAL does not precipitate anaphylactic reactions and that the use of BLFC six weeks following Bacillus Calmette–Guérin (BCG) instillation does not increase the rate of false positivity [4, 6, 14]. At this time, the primary limitations barring increased utilization of this modality are cost and clinic workflow inefficiencies.

Though NBI and BLC have demonstrated increased sensitivity for the detection of CIS and multifocal papillary lesions, the data supporting BLC is more robust. BLC has a number of prospective, randomized trials, whereas) the majority of data supporting NBI is retrospective. This discrepancy in the quality of data is reflected in the AUA/SUO recommendations, which list the use of BLC as a moderate recommendation and the recommendation of NBI being conditional. Randomized, prospective trials evaluating NBI would be helpful in interrogating its efficacy.

Near-Infrared Fluorescence Imaging

Like BLC and NBI, near-infrared (NIR) fluorescence conveys images through the reflection of light at a specific wavelength, 650–900 nm [11]. Light that is emitted in the NIR spectrum has deeper tissue penetration and less background fluorescence of adjacent tissues when compared to visible-range light owing to decreased hemoglobin and water absorption [10, 11]. These characteristics make NIR fluorescence imaging favorable for cystoscopic evaluation.

Porphyrins, which are more highly , which are more highly concentrated in malignant tissues than benign tissues, are endogenous fluorophores of interest when utilizing NIR [10]. When visualizing the autofluorescence (AF) signal of benign tissues compared to malignant tissues, benign tissues have a higher AF signal when excited by identical wavelengths [10]. The AF of benign tissues is brighter than the AF seen in malignant tissues [10]. Therefore, tumors will appear muted on a brighter background [10]. Important exceptions are necrotic tumors and prior resection sites, which may be secondary to an increase in porphyrins in more advanced tumors or the presence of different biochemically active molecules [10]. Therefore, when NIR is used to evaluate a prior resection site, the same increased AF is appreciated [10]. There are currently investigations into probes that can be used simultaneously NIR specific for bladder cancer [10] and probes that can be cleaved by intracellular proteases while the excess is excreted from the body [11].

AUA/SUO Guidelines

The current AUA/SUO guidelines make a moderate recommendation for the use of blue light cystoscopy in patients with non-muscle-invasive bladder cancer at the time of transurethral resection of bladder tumors if the technology is available, citing the evidence strength as Grade B. In the same patient population, the conditional recommendation for the use of NBI is made, citing the evidence strength as Grade C. For both types of cystoscopy, the justification for both is evidence demonstrating their abilities to increase detection and decrease recurrence [3, 14].

Future Directions

There is preliminary data suggesting that Cysview in combination with blue light may be cytotoxic. The accumulation of protoporphyrin IX in urothelial cancer cells is cytotoxic when irradiated with white light following the instillation of HAL and utilization of BLC for TURBT [14]. This experimental methodology yielded 6-month, 9-month, and 21-month preliminary efficacies of approximately 52%, 23%, and 11%, respectively, in patients with intermediate- or high-risk bladder cancer [1, 14]. These technologies warrant further exploration.

Conclusion

WLC is the current standard of care for bladder cancer surveillance. Though it is an invasive outpatient procedure, there are a number of low-cost strategies to increase tolerability. Current data supports the ability of enhanced cystoscopy to facilitate a more complete resection of bladder tumors with BLC and NBI-assisted TURBTs.

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Chapter 9
Urine Cytology in the Clinical Management of Bladder Cancer

Andrew T. Gabrielson, Christopher J. VandenBussche, and Max R. Kates

Introduction

The examination of urine is one of the oldest medical procedures dating back to ancient Egypt due to the ease by which urine could be obtained and the direct correlation of macroscopic examination (uroscopy) to various disease states [1]. While the pathophysiologic processes behind changes in urine were not completely understood at that time, alterations in the gross appearance of urine were often the first indicators of disease. The observation of ants attracted to open urine containers was an early method to identify those with diabetes mellitus. The invention of the *matula*, a circular glass flask into which patients would urinate, allowed uroscopists to carefully assess the color and quality of the urine.

The first microscopic examination of the cells in urinary sediment was reported by the Czech doctor Lambl in 1856 [2]. Urinary tract cytology (UTC)) was further popularized by Papanicolaou, the father of cytology, who utilized his Pap stain to better examine urinary tract specimens under the microscope [3]. Finally, Koss made numerous significant contributions to the field of UTC, better defining cytomorphological findings in the context of the histopathologic classification of bladder cancers during that era [4].

The utility of UTC relies heavily on the discohesive nature of high-grade urothelial carcinoma (HGUC)) as well as carcinoma in situ (CIS). While washing (barbotage) can forcibly exfoliate normal urothelial lining as well as neoplastic cells of any grade, most HGUC/CIS (together called "HGUC" for the rest of this section)

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cells have alterations which increase their natural exfoliation into urine. The same is not true for low-grade urothelial neoplasm (LGUN)) such as low-grade urothelial carcinoma. Therefore, voided UTC specimens contain naturally exfoliated cells and can detect HGUC cells that have arisen anywhere in the urinary tract, whereas selective cytology (washing) specimens contain benign urothelial fragments as well as low- and high-grade urothelial carcinoma cells, when present.

Urinary Tract Cytomorphology and the Paris System for Reporting Urinary Cytology (TPS)

Various benign cellular and acellular components can be found in a UTC specimen. These include benign urothelial, squamous, renal tubular, and glandular cells, bacterial and fungal organisms, red blood cells, inflammatory cells, crystals, and casts (Fig. 9.1). Less commonly spermatozoa, corpora amylacea, and seminal vesicle cells may be seen (Fig. 9.2). Voided UTC specimens may be contaminated by extraurinary components, such as endometrial cells and squamous cells. Instrumented (washing, brushing, and catheterized) specimens are more likely to contain urothelial tissue fragments; however, benign urothelial tissue fragments may be seen voided UTC specimens in the setting of (clinical and subclinical) urolithiasis (Fig. 9.1d).

Malignant and atypical cells seen in UTC specimens may arise from the urothelium or prostate, invade into the urinary tract from adjacent organs, or contaminate the specimen from the gynecologic tract or external genitourinary regions (Fig. 9.3). Cytomorphological examination can sometimes determine a likely site of origin based on cellular differentiation (e.g., squamous, glandular, or urothelial) as well as certain cytomorphological characteristics (e.g., endometrial vs. colorectal carcinoma). Since HGUC is by far the most commonly identified malignancy in UTC specimens, the discussion that follows primarily focuses on the diagnosis of HGUC.

Cells derived from papillary HGUC as well as CIS lesions have similar cytomorphological features, and thus, these lesions cannot be distinguished using UTC. UTC is also unable to distinguish between invasive and noninvasive HGUC, as well as whether HGUC cells are derived from the upper or lower urinary tract. Due to their discohesive biology, HGUC cells are often present singly rather than in tissue fragments, especially in voided urine specimens. The number of cells may be limited in voided urine specimens or when the cells are derived from CIS lesions. By contrast, a washing procedure directed at a papillary urothelial neoplasm typically yields a larger number of neoplastic cells.

Compared to normal intermediate (parabasal-like) urothelial cells, HGUC cells are larger and have larger nuclei, higher nuclear-to-cytoplasmic (N/C) ratios, irregular nuclear contours, dark chromatin (hyperchromasia), and greater variation in nuclear size (anisonucleosis). One important and relatively specific feature is an atypical chromatin pattern; HGUC cells typically have irregularly clumped (coarse)

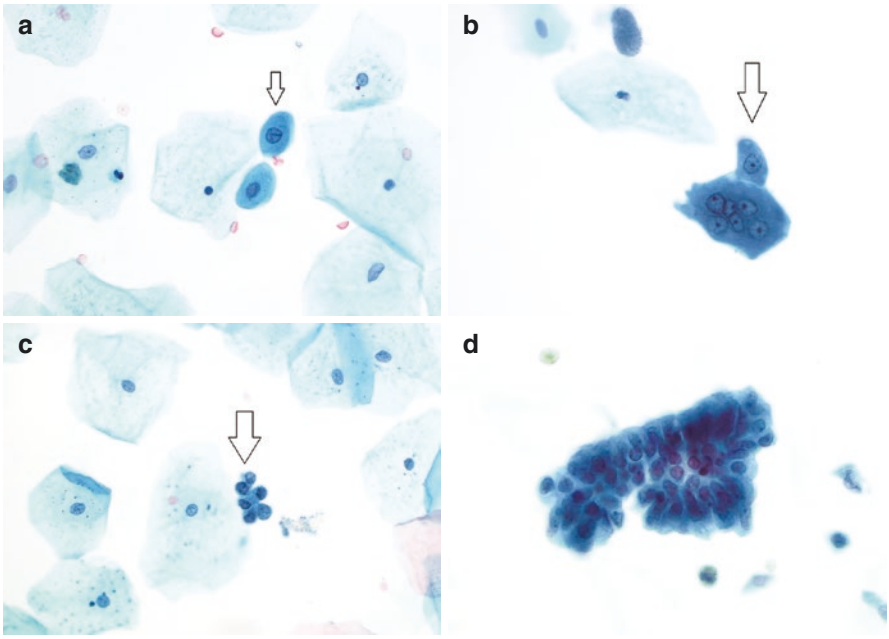

Fig. 9.1 Benign components found in urinary tract cytology. (**a**) Intermediate ("parabasal-type") urothelial cells look like fried eggs, with oval-shaped nuclei, regular nuclear contours, and a bland chromatin pattern. They naturally exfoliate into voided urine specimens and are usually seen as single cells. Nuclear-to-cytoplasmic ratios are below 0.5. (**b**) Umbrella cells are the most superficial cells lining the urinary tract. They have abundant, granular cytoplasm, small nucleoli, and a condensed rim of chromatin around the nuclear border; they may be multinucleated. (**c**) Renal tubular cells may be seen in voided urine specimens. They form rare, small groups of loosely cohesive cells. They often have high nuclear-to-cytoplasmic ratios, dark nuclei, and irregular nuclear contours, which may cause concern for malignancy if they are not recognized as renal tubular cells. (**d**) The field shows a benign urothelial tissue fragment. The nuclei are evenly arranged within the fragment, are approximately the same size as one another, and have a bland chromatin pattern. Tissue fragments are more frequently seen in washing (barbotage) specimens than in voided urine specimens; the differential diagnosis includes fragments from a low-grade urothelial neoplasm, which cannot always be distinguished from benign urothelial cells in urinary cytology

chromatin that is unevenly distributed within the nucleus (Fig. 9.4). Rarely HGUC cells may have prominent nucleoli rather than coarse chromatin; neoplasms composed of paradoxically hypochromatic cells are also occasionally seen (Fig. 9.4d). Other features associated with HGUC include cell cannibalism (a "cell-in-cell" appearance) and the presence of intracytoplasmic lumens (ICLs) (Fig. 9.5c).

Histologic variants of HGUC have a similar appearance as conventional HGUC, with the exception of the micropapillary variant, which has a glandular appearance in UTC and may cause concern for a primary or secondary adenocarcinoma. Histologic variants which are associated with infiltrative cells (such as the plasmacytoid variant) may be more difficult to detect by UTC, as the cells infiltrate into the

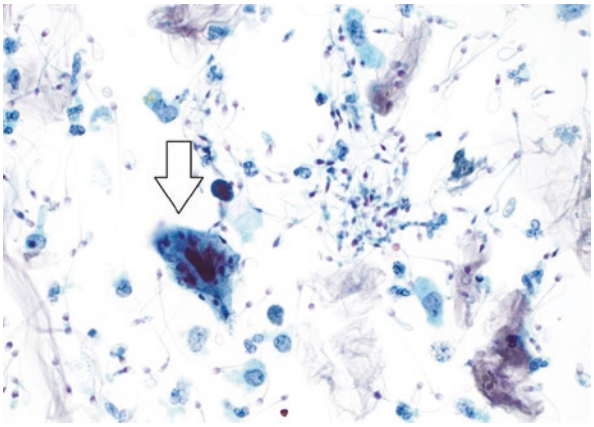

Fig. 9.2 Seminal vesicle cell (arrow) in the background of spermatozoa. Seminal vesicle cells may rarely be seen and are usually associated with spermatozoa. Seminal vesicle cells are large with abundant cytoplasm and are most easily identified by the presence of cytoplasmic golden pigment (not seen in this case). They may have large, highly atypical nuclei and cause concern for malignancy

tissue rather than forming a mucosal mass of exfoliating tumor cells. Finally, HGUC with squamous, glandular, or small-cell components have distinctive cytomorphology and may raise the differential diagnosis of a pure squamous cell carcinoma, adenocarcinoma, or small-cell carcinoma, either primary to or secondarily involving the urinary tract (Fig. 9.4f).

When well-preserved HGUC cells are present, the diagnosis is usually straightforward. However, HGUC cells are rarely well-preserved, as they begin to degenerate as they sit in the bladder at body temperature. Urine is also a poor matrix for cellular preservation. If specimens are not immediately fixed, processed, or refrigerated after collection, cells will continue to degenerate at room temperature. During degeneration, generally, a cell's nucleus will condense, and the cell's cytoplasm will become vacuolated. This causes a decrease in the N/C ratio and makes the assessment of chromatin patterns difficult (Fig. 9.5). These alterations may result in a specimen being classified into an indeterminate category (such as "atypical" or "suspicious"). As cells further degenerate, the nuclear-cytoplasmic interface becomes indistinct, and it becomes difficult to distinguish between degenerated HGUC cells and degenerated benign urothelial cells. Thus, degenerated benign cells may cause an otherwise "negative" specimen to be classified into an indeterminate category. Finally, treatment with immunotherapy, chemotherapy, and/or radiation therapy can result in cellular changes to both benign and malignant cells; these changes may contribute to increases in indeterminate diagnoses (Fig. 9.5d) [5].

LGUNs have bland cytomorphology compared to HGUC, and the cells, when present in a specimen, have significant overlap with benign urothelial cells. Because of this overlap, it is unclear how frequently LGUN cells naturally exfoliate into voided urine specimens. However, studies have consistently shown the diagnosis of

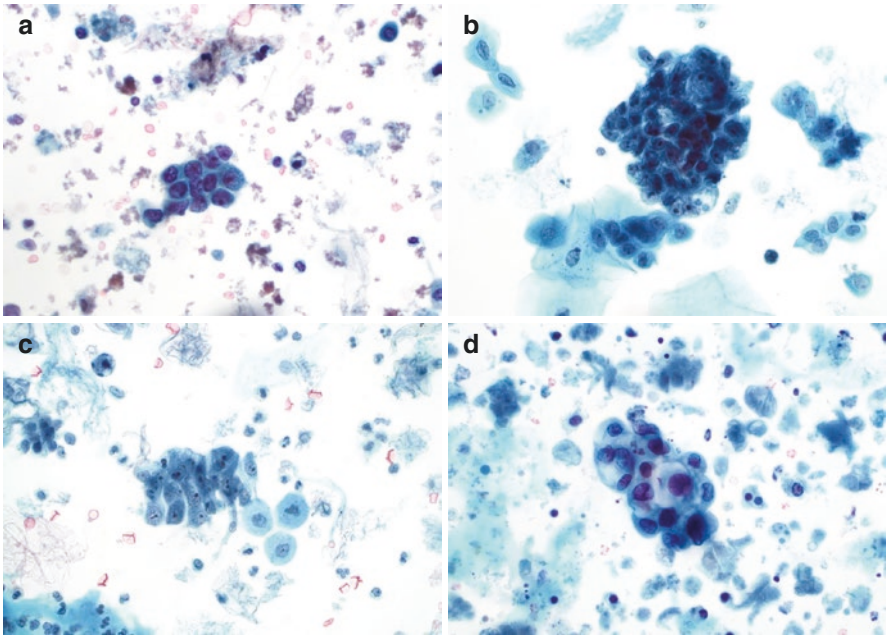

Fig. 9.3 Non-urothelial malignancies. (**a**) Prostate carcinoma. This small fragment contains cells with high nuclear-to-cytoplasmic ratios and prominent nucleoli. Prostate carcinoma, renal cell carcinoma, and melanoma cells all typically have prominent nucleoli. High-grade urothelial carcinoma (HGUC) can sometimes have prominent nucleoli but usually demonstrates a coarse (clumpy) chromatin pattern. (**b**) Compared to HGUC, adenocarcinomas are more likely to have prominent nucleoli and form three-dimensional tissue fragments. Following a tissue biopsy and immunohistochemical studies, this patient was found to have an intestinal-type adenocarcinoma, a situation that requires clinical correlation to determine whether the malignancy is primary to the bladder or arose from the gastrointestinal tract. (**c**) A separate case of intestinal-type adenocarcinoma. The cells have a columnar shape, which correlates to their glandular differentiation. (**d**) Metastatic pancreatic cancer. This patient had widely metastatic pancreatic adenocarcinoma. In contrast to what would be typically seen with HGUC, these cells form a cohesive fragment and have abundant, vacuolated cytoplasm

LGUN to have low sensitivity, specificity, and reproducibility. Washing procedures directed at papillary LGUNs usually result in a hypercellular specimen with a monotonous population of small cells with oval-shaped, eccentrically placed nuclei and N/C ratios that approach 0.5 [6]. The neoplastic cells have regular nuclear contours and a bland chromatin pattern, often with only a small nucleolus; this chromatin pattern differs significantly from the coarse pattern seen in HGUC (Fig. 9.6). True papillary fragments possess fibrovascular cores and may rarely be seen, usually with neoplastic cells attached and dispersed in the background. However, fibrovascular cores may also be seen in papillary HGUC and are therefore a nonspecific finding. Histologically, LGUN lesions may contain focal high-grade areas that may or may not also be present in cytology specimens; it is uncertain the degree to which

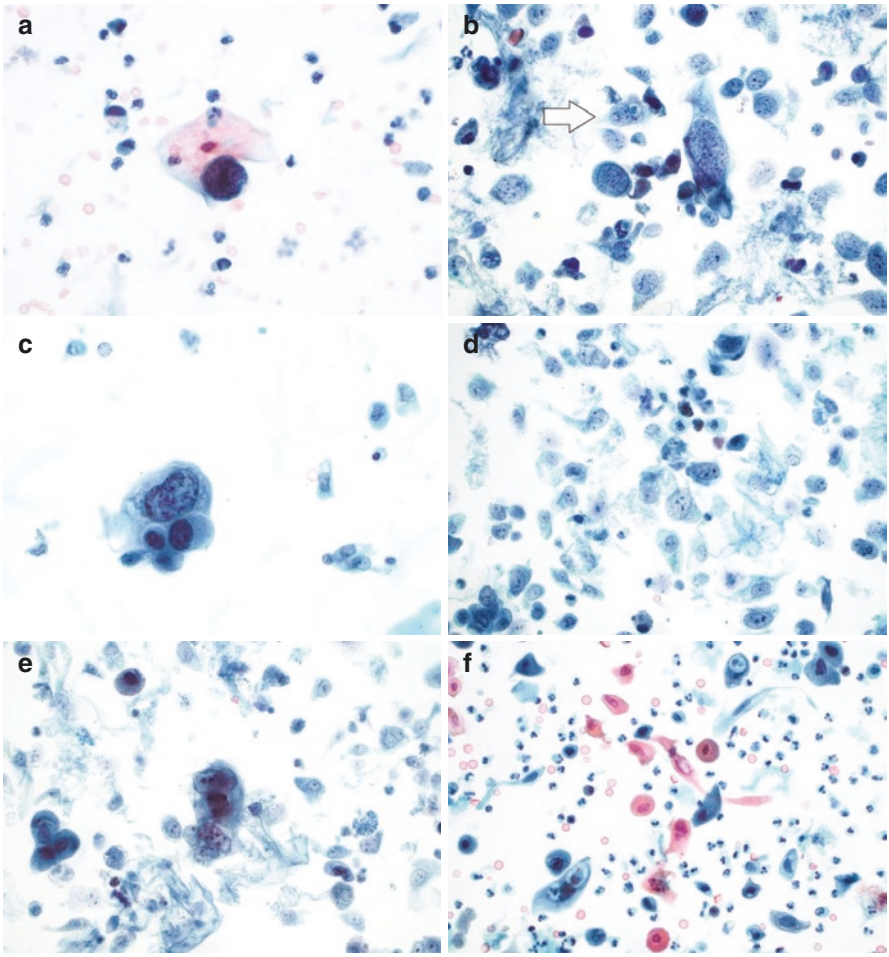

Fig. 9.4 High-grade urothelial carcinoma (HGUC). (**a**) This well-preserved cell demonstrates the features of HGUC: hyperchromasia (dark chromatin), irregular nuclear borders, high nuclear-to-cytoplasmic ratio (only a thin rim of blue cytoplasm can be seen), and coarse (clumpy) chromatin. (**b**) Numerous, singly dispersed HGUC cells are seen in this field, which demonstrates how variable HGUC cells can look even within the same specimen. Note the great variation in nuclear size (anisonucleosis), high nuclear-to-cytoplasmic ratios, and distinctive coarse chromatin. One cell (arrow) has an intracytoplasmic lumen containing condensed cyanophilic (blue) material, a feature seen in more aggressive HGUCs. (**c**) While HGUC cells are usually discohesive, these HGUC cells are forming a small fragment which demonstrates the variation in nuclear size and shapes. While the larger cell is overtly malignant, other cells with lower nuclear-to-cytoplasmic ratios may not be sufficient for a diagnosis of HGUC on their own. (**d**) Most of the cells in this field are HGUC cells, as identified by their large size, high nuclear-to-cytoplasmic ratios, and coarse chromatin. However, their nuclei are more pale (hypochromatic) than dark, which makes them less concerning at first glance. (**e**) The large cell in the center is multinucleated and has abundant cytoplasm. In some instances, this morphology may overlap with reactive umbrella cells. However, the distinctly coarse chromatin seen here is diagnostic of malignancy. (**f**) HGUC may have a component of squamous differentiation, as seen here by the pink-staining cells with irregular shapes. The conventional urothelial component may not be seen in urinary tract cytology specimens; thus, the differential diagnosis also includes a primary squamous cell carcinoma, a metastatic squamous cell carcinoma, and a squamous cell carcinoma invading from adjacent organs (e.g., cervix)

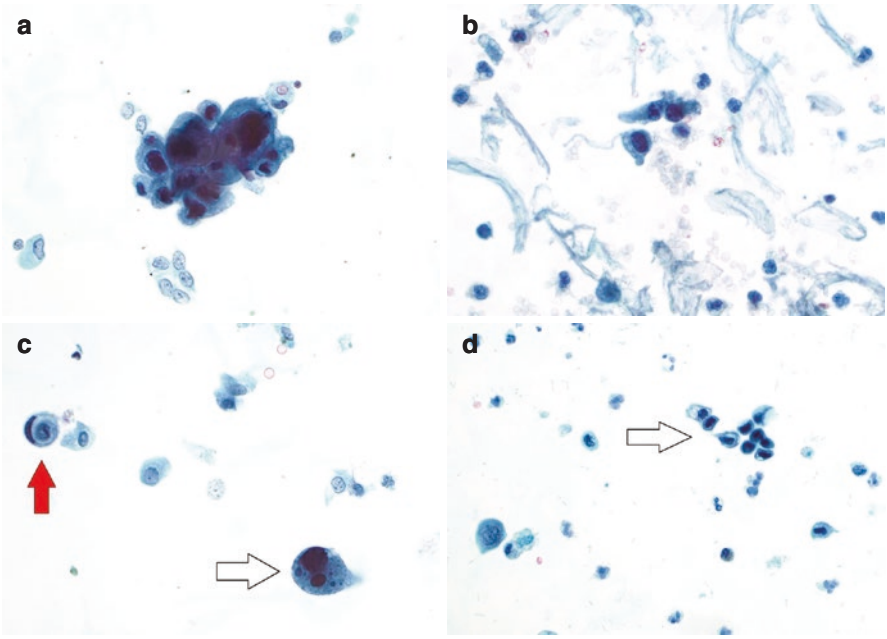

Fig. 9.5 Degeneration. (**a**, **b**) HGUC cells often become degenerated before cytologic preparations are made. The cells will have condensed (pyknotic) nuclei and more abundant, vacuolated cytoplasm. In these two separate cases, the nuclei are sufficiently large and dark that a diagnosis of malignancy can still be made. (**c**) This is voided urine from a patient with HGUC. The bottom cell (white arrow) has a cytoplasm filled with condensed organelles, and the nucleus is indistinct; thus, this cell cannot be assessed to determine the presence of malignancy. The specimen also shows cell cannibalism (red arrow) which is usually associated with HGUC but is not sufficiently specific on its own to allow for such a diagnosis. (**d**) This group of small cells (arrow) are benign degenerated urothelial cells. The nuclei are also condensed and dark but are smaller than the nuclei of the surrounding benign bystander cells. Such changes can have overlap with degenerated HGUC and may result in an indeterminate diagnosis

this phenomenon may cause discrepancies between cytology and histologic diagnoses.

While several attempts have been made to standardize the assessment of UTC, the first system to gain wide acceptance has been The Paris System for Reporting Urinary Cytology (TPS) [7]. TPS working groups were formed following the discussion of UTC at the 2013 International Congress of Cytology in Paris. The discussion centered around the need for improvement in UTC, with concern that the high rate of indeterminate diagnoses in many laboratories was greatly reducing the clinical utility of UTC. In the years that followed, the working groups conducted an extensive review of the literature, surveyed pathologists around the globe, and sought urologist input.

The first iteration of TPS was released in 2016 and was accompanied by a fascicle containing representative photographs, comprehensive literature reviews, and expert recommendations regarding the approach and assessment of UTC

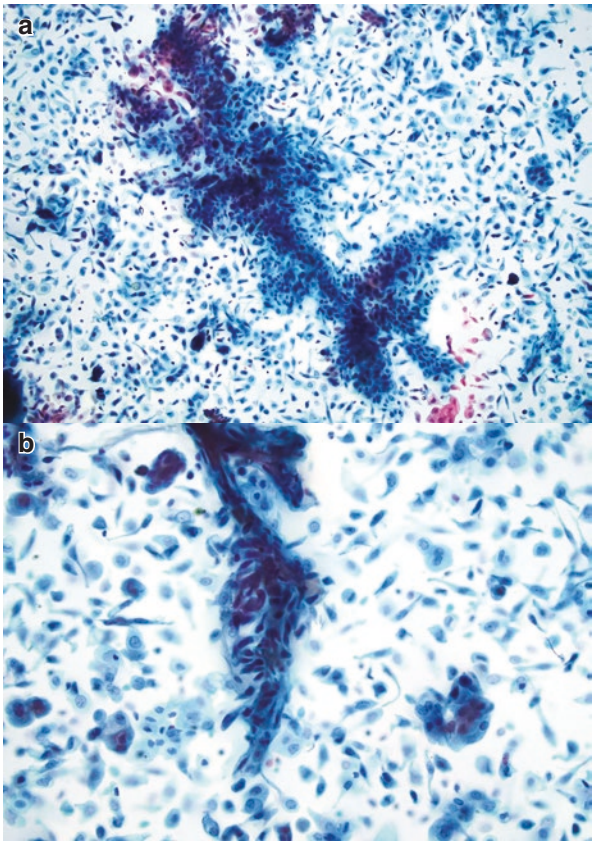

Fig. 9.6 Low-grade urothelial carcinoma. (**a**, **b**) Low-grade urothelial neoplasms can typically only be identified in washing (barbotage) specimens in which "true" papillary fragments (those that contain fibrovascular cores) are seen with associated neoplastic cells. The background may be cellular with a monotonous population of neoplastic cells with oval nuclei and bland chromatin. Long cytoplasmic tails may be seen ("cercariform cells"). In forming a diagnosis, it is most important for the pathologist to identify any cells concerning HGUC

specimens. TPS not only provides specific nomenclature for its diagnostic categories but also defines specific cytomorphological features defining each diagnostic category (Table 9.1). The primary cytomorphological features used by TPS for a diagnosis of HGUC are hyperchromasia, nuclear contour irregularities, coarse chromatin pattern, and increased N/C ratio.

TPS focuses on maintaining the high-specificity UTC for HGUC diagnoses while reducing the number of indeterminate diagnoses. This is accomplished in two ways. First, the criteria for an HGUC diagnosis are stringent, such that a diagnosis of HGUC has a high positive predictive value for HGUC. Secondly, TPS discourages a diagnosis of LGUN using UTC alone, as reflected by the name of the "negative" category ("negative for HGUC" (NHGUC)). Thus, a diagnosis of NHGUC indicates that features of HGUC were not found in a given specimen, but a diagnosis of NHGUC does not exclude concern for LGUN. This practice was expected to greatly reduce the number of indeterminate categories since some pathologists would previously use indeterminate categories when encountering mildly atypical urothelial cells that, while not concerning for HGUC, could possibly represent LGUN.

When a definitive diagnosis of HGUC cannot be made but cells concerning for HGUC are present, one of two TPS categories may be used. "Suspicious for HGUC"

(SHGUC) is the higher-risk category and "atypical urothelial cells" (AUC) is the) lower-risk category. As of yet, there are no standardized clinical guidelines for managing patients with an AUC or SHGUC diagnosis, and urologists should use their best judgement to manage these patients, taking into account a patient's overall risk of disease. Due to the high PPV of SHGUC for HGUC on follow-up, a more

Table 9.1 The Paris system for reporting urinary cytology

Diagnostic category	Description	ROHM[a]
Negative for high-grade urothelial carcinoma (NHGUC)	No features of high-grade urothelial carcinoma are identified	11–27%
Atypical urothelial cells (AUC)	Some qualitative features of high-grade urothelial carcinoma are identified in at least one urothelial cell TPS criteria: Nuclear-to-cytoplasmic ratio at or above 0.5 The specimen does not meet the criteria for SHGUC or HGUC One additional atypical feature: Hyperchromasia Irregular nuclear contours Coarse (clumpy) chromatin	33–59%
Suspicious for high-grade urothelial carcinoma (SHGUC)	Marked urothelial atypia is seen, but the specimen does not meet either the qualitative or quantitative criteria for the HGUC category TPS criteria: Nuclear-to-cytoplasmic ratio at or above 0.5 The specimen does not meet the criteria for HGUC Two additional atypical features: Hyperchromasia Irregular nuclear contours Coarse (clumpy) chromatin Note: Specimens with overtly malignant cells may be classified as SHGUC if the number of cells is insufficient for a diagnosis of HGUC Specimens may contain numerous markedly atypical cells and be classified as SHGUC if the amount of atypia falls between that seen in the AUC and HGUC categories	76–93%
High-grade urothelial carcinoma (HGUC)	A sufficient quantity of overtly malignant cells can be identified TPS criteria: Nuclear-to-cytoplasmic ratio at or above 0.7 The following atypical features are seen: Hyperchromasia Irregular nuclear contours Coarse (clumpy) chromatin Note: TPS recommends identifying at least 5 malignant cells in voided urine specimens and bladder washing specimens and at least 10 malignant cells in upper tract washing/brushing specimens.	89–100%

(continued)

Table 9.1 (continued)

Diagnostic category	Description	ROHM[a]
Low-grade urothelial neoplasm (LGUN)	"True" papillary fragments containing fibrovascular cores associated with a monotonous population of neoplastic cells with bland chromatin, regular nuclear contours, and low (<0.5) nuclear-to-cytoplasmic ratios Notes: Typically only seen in washing (barbotage) specimens The diagnosis can only be made in the appropriate clinical setting (e.g., a tumor is seen on cystoscopy) If any features of HGUC can be identified, the AUC, SHGUC, or HGUC categories should be used instead Most cytopathologists will not utilize this category and make a diagnosis of NGHUC instead	N/A
Nondiagnostic (unsatisfactory)	Limitations in the specimen raise concern for a false-negative diagnosis. Limitations include small voided urine specimen volume, low numbers of urothelial cells in washing/brushing specimens, and the presence of obscuring factors (e.g., bacteria, inflammatory cells, crystals) Notes: This category is not standardized due to limited evidence in the literature Evidence indicates that voided urine specimens with volumes below 25 cc have a decreased sensitivity for detecting HGUC Evidence indicates that washing specimens with low numbers of urothelial cells have a decreased sensitivity for detecting HGUC.	N/A

[a]Unpublished data are taken from the forthcoming revision of TPS (personal communication with Drs. Mauro Saieg and Ricardo Pastorello, Santa Casa de São Paulo School of Medical Sciences, Brazil. The data is from five published studies from institutions following their implementation of TPS. The rate of high-grade malignancy (ROHM) is calculated from specimens with concurrent or follow-up biopsies and thus likely overestimates true ROHM

aggressive follow-up is generally indicated. However, it is not currently recommended that a diagnosis of SHGUC alone be used for definitive treatment. It is uncertain whether the AUC category will become sufficiently predictive of HGUC to merit a change in patient management. It is possible that the AUC category may best be used in combination with ancillary testing, where an ancillary test is reflexively ordered on AUC specimens and the test result used to guide clinical management.

Studies since the 2016 TPS have shown that TPS reduces atypia rates in the laboratory by approximately 50% [8]. Due to the stringent criteria for an HGUC diagnosis, some laboratories have shown a slight shift of specimens from the HGUC to SHGUC categories. In laboratories that have implemented TPS, the mean risk of HGUC among patients with follow-up biopsy was 65% for the AUC category and 84% for the SHGUC category (unpublished data). The risk of HGUC among patients with follow-up biopsy was 89–100% for the HGUC category and 9–30% for the NHGUC category (unpublished data).

A revised version of TPS ("TPS 2.0") is forthcoming. Although TPS 2.0 will not make significant alterations to the system, it will address many specific questions and problems which arose from the first version. Importantly, it will include an expanded set of criteria for diagnosing HGUC with the intention of increasing sensitivity without sacrificing specificity. TPS 2.0 will also include data on the performance of TPS since its implementation and address special topics, such as upper tract urothelial carcinoma and non-urothelial lesions sometimes seen in UTC specimens.

Urine Cytology in Clinical Practice

The utility of UTC in the workup and management of UC of the bladder continues to evolve, particularly with the advent of advanced imaging techniques such as computerized topography (CT) with delayed-phase imaging. In the subsequent sections, we will highlight methods of obtaining and processing UTC, performance characteristics with respect to detecting LGUC and HGUC, and specific indications to use (or avoid) UTC depending on the clinical circumstance.

Methods of Obtaining Urine Cytology

There are two main methods of obtaining UTC for detection of clinically significant UC of the bladder. A washing or barbotage specimen may be obtained by placement of a urinary catheter and vigorously irrigating the bladder with saline solution. Alternatively, a barbotage specimen may be obtained during cystoscopy, in which the cytology sample is obtained after the bladder is filled with irrigation. Early studies have demonstrated that bladder washing cytology yields more tumor cells in a sample and is more sensitive in identifying clinically significant cancer, particularly high-grade UC [9]. One downside of obtaining bladder washings or barbotage specimens is that the act of instrumentation into the bladder may potentiate reactive cellular changes, which may contribute to variability in pathologic interpretation. One study found that urine obtained after instrumentation decreased the specificity of UTC for UC [10]. Other studies have confirmed that flexible cystoscopy may increase urothelial cell count, potentiate nuclear atypia, and lead to the formation of papillary aggregates, which may contribute to misinterpretation of urine samples [11]. Nonetheless, other studies have shown instrumentation had no impact on UTC performance characteristics [12].

An alternative method of obtaining UTC involves obtaining a voided urine specimen, typically at least 20 mL. Although voided specimens are less invasive, as cystoscopy and catheter placement are not required, there are several caveats to using this method. Specimens should not be obtained from the first void of the day as cells sitting dependent within the bladder overnight may appear abnormal histologically

and complicate interpretation of the specimen. Additionally, patients producing an abnormally large volume of urine may prevent an adequate concentration or a total number of cells from being obtained for a proper cytologic examination.

For patients with concomitant bladder and upper tract UC, various contrast agents may be used intraoperatively to define anatomy and identify filling defects consistent with a mass. Traditional dogma dictates that bladder or upper tract washings collected for cytological analysis should be performed without interference from contrast agents which may alter cellular integrity and diagnostic interpretation [13]. However, a study demonstrated that commonly used contrast agents do not alter urothelial cell morphology at exposures up to 5 minutes prior to fixation with formalin or methanol [13]. Regardlessfixation with formalin or methanol of whether the urothelial cells were washed with contrast or saline, no differences were observed in cellular morphology or in the pathologist's ability to correctly differentiate benign from malignant cytology [13]. These results suggest that contrast media do not confound cytological interpretation of upper tract washings and do not need to be discarded as long as fixation occurs within 5 minutes following collection.

UTC may also be obtained in the form of urethral washings [14]. This is particularly relevant to patients who have undergone radical cystectomy with urinary diversion either in the form of an ileal conduit with urethral preservation or an orthotopic neobladder and are being surveilled for local recurrence [15]. Several large series have demonstrated that the risk of UC within a retained urethra following urinary diversion may be as high as 17% [16, 17]. As such, patients with risk factors for the development of urethral recurrences following diversion (presence of CIS, tumor multifocality, tumors at the bladder neck, prostatic urethra, or stromal involvement) should be monitored with occasional urethra wash cytology [18, 19].

Specimen Preparation and Adequacy Assessment

UTC specimens may be prepared using several methods. Urine may be centrifuged to form a pellet which is then smeared across a glass slide ("conventional smear"). This method is work-intensive and preserves acellular material, blood, inflammation, and large tissue fragments, all of which can obscure the cells of interest and limit diagnosis. Alternatively, cells may be directly centrifuged onto a glass slide (cytocentrifugation). Cytocentrifugation is particularly useful for paucicellular specimens (such as voided urines) as the specimen is concentrated into a small area rather than smeared across an entire glass slide. The Millipore method consists of urine passed through filter paper, which is subsequently pressed onto a glass slide, allowing the transfer of trapped cells. This method allows cells of interest to be preserved while eliminating small and large obscuring elements.

Liquid-based preparations (LBPs; e.g., ThinPrep or SurePath) were originally developed to prepare cervical (Pap test) specimens. LBPs use various proprietary technologies to eliminate small and large obscuring factors, automate and standardize preparation, and disperse cells evenly within a limited area on a glass slide.

The volume of urine received is pelleted and then resuspended in a small amount of alcohol-based preservative before being processed. Alternatively, a fixative solution may be added to freshly collected urine before the specimen is transported to the laboratory. LBPs are more expensive than the traditional methods described above but are the most reliable in producing good-quality specimens, which reduces the time needed for slide review and, presumably, the number of suboptimal diagnoses.

Since cytology specimens typically contain a small number of cells when compared to tissue biopsy, an adequacy assessment usually accompanies all cytologic diagnoses. An inadequate specimen is considered non-informative and does not exclude the presence of disease. There are only a few published studies on adequacy in UTC specimens, and thus, adequacy criteria are not well defined. In theory, the adequacy of a voided urine specimen depends on the volume submitted, with some studies indicating that volumes below 25 cc are associated with decreased sensitivity for the detection of HGUC [20]. While some pathologists may consider voided urine specimens of low cellularity to be inadequate, well-hydrated patients may produce a high-volume, low-cellularity specimen. Alternatively, some pathologists may simply note that these specimens contain a scant urothelial cell component rather than diagnosing them as inadequate. In theory, the frequency by which malignant cells are found in UTC specimens should correlate with disease burden; if true, this would suggest the collection of serial UTC specimens could increase test sensitivity.

Performance Characteristics of Urine Cytology in Detecting Clinically Significant UC of the Bladder

In the diagnosis of UC of the bladder, urine cytology has consistently been shown to be a low-sensitivity and high-specificity test. A total of 12 studies and one systematic review and meta-analysis including over 2000 patients have evaluated the diagnostic characteristics of urine cytology [21]. Pooled analyses were performed using data derived from 9 of the 12 studies. In the studies, “inconclusive” cytology was excluded, and “atypical” was considered negative. The pooled sensitivity and specificity of urine cytology in the meta-analysis was 20% (95% CI 2.5–72%) and 99.8% (95% CI 94–100%), respectively [21].

It is important to note that the sensitivity and specificity characteristics for the aforementioned studies lump both LGUC and HGUC of the bladder together. The sensitivity of urine cytology, which depends largely on the degree of tumor differentiation, is markedly different in detecting low-grade versus high-grade disease [22, 23]. HGUC which often demonstrates severe nuclear pleomorphism and hyperchromasia is identified more accurately than low-grade tumors [22]. Low-grade tumors are less likely to exfoliate cells into the bladder due to preserved intercellular attachments and, thus, the yield of cytology may be poor [23]. This results in a high

false-negative rate (10–50%) and thus poor sensitivity in low-grade and early-stage UC. Due to these data, the AUA currently does not recommend using urine cytology in surveillance of LGUC of the bladder [24].

On the contrary, the sensitivity of urine cytology for the detection of HGUC and carcinoma in situ (CIS) is significantly higher than LGUC [25]. One prospective cohort study enrolling patients with microscopic and gross hematuria found that the diagnostic sensitivity of cytology was 57.7% (95% CI 38.7–75.3%) for high-risk tumors [25]. The sensitivity of cytology in detecting CIS varies from 66% to 83% [26–28]. One study conducted in 592 bladder washing samples, including 50 patients with CIS, found the diagnoses of either "suspicious for high-grade neoplasia" or "consistent with high-grade neoplasia" to be 70% sensitive and 99% specific for CIS [26].

Another important factor that may affect the sensitivity of UTC is the expertise of the pathologist. One study conducted in 1034 patients undergoing cystoscopy and UTC over a four-year period found that the value of UTC is impacted by the individual learning curve. The sensitivity for low-grade tumors decreased significantly from 86% to 56% over a four-year learning curve of a local cytopathologist at the beginning of the learning period. However, the sensitivity of high-grade tumors remained constant (86–77%). Specificity significantly increased over the four-year learning curve from 66% to 84%. This study reflects that pathologists of varying experiences will be effective at identifying clinically significant (high-grade) cancers without a steep learning curve [29].

Urine Cytology in the Diagnostic Evaluation of Hematuria

Urine cytology has been recommended and is widely used in surveillance of UC of the bladder; however, there is no consensus among guideline bodies regarding the use of UTC for the evaluation of microscopic hematuria [30–32]. The AUA currently does not recommend the use of urine cytology or urine-based tumor markers in the initial evaluation of microscopic hematuria. The most recent 2020 microscopic hematuria guidelines provide a risk-stratified approach based on the patient's age, smoking history, and the degree of microscopic or gross hematuria [30]. Patients that are deemed intermediate or high-risk based on the AUA risk stratification should undergo cystoscopy with either renal ultrasound or CT urography. In order for UTC to be considered valuable in this setting, it would need to demonstrate added benefit in tandem with cystoscopy. Currently, no data supports this notion. One prospective study which included 2778 patients with microscopic hematuria found that only two patients (0.07%) with negative cystoscopy, ultrasound, IV pyelogram, and positive cytology were ultimately diagnosed with UC [33]. This study also demonstrated a 10.5% false-positive rate which ultimately leads to unnecessary evaluations and follow-up. In another large cohort study, 567 of 3556 patients presenting with microscopic (30.3%) or gross hematuria (69.7%)

underwent urine cytology in the initial workup for UC [25]. Not a single case of bladder UC or upper tract UC was diagnosed based on UTC alone. All in all, the evidence to support the use of cytology in the diagnostic evaluation of microscopic hematuria is poor and likely leads to more costly and unnecessary follow-up care. The only circumstance where UTC may be beneficial in the setting of microscopic hematuria is in patients with persistent microscopic hematuria who have irritative lower urinary tract symptoms and other risk factors for CIS. However, it is important to note that this recommendation is considered expert opinion and has not been rigorously tested prospectively.

Although UTC should not play a role in the workup for patients with microscopic hematuria, the AUA currently does not provide a recommendation about whether or not to utilize UTC in the workup of macroscopic or gross hematuria [24]. Nonetheless, it is reasonable to consider using UTC in this patient population, particularly given the significantly higher pretest probability (23% vs. 4%) of identifying an underlying urothelial cancer compared to patients with microscopic hematuria [34].

Urine Cytology for Surveillance of UC of the Bladder

Today, the most common use for urine cytology is in the surveillance of patients with a history of non-muscle-invasive bladder cancer, particularly those with a history of high-grade disease and CIS [35]. Based on the current AUA guidelines, for those patients with a history of low-risk non-muscle-invasive disease, cytology should not be performed. For patients with intermediate-risk disease, cystoscopy and urine cytology should be performed every 3–6 months for 2 years, then 6–12 months for years 3 and 4, and annually thereafter [35]. For patients with high-risk diseases, cystoscopy and cytology should be performed every 3–4 months for 2 years, every 6 months for years 3 and 4, then annually thereafter [35]. The recommendations for both intermediate-risk and high-risk patients are both based on expert opinion and are highly variable even among centers of excellence.

One diagnostic dilemma of urine cytology is the case of positive cytology (suspicious for or presence of HGUC) with a negative cystoscopy [36]. In this circumstance, further evaluation is warranted of the upper urinary tracts. This may involve upper tract washings or barbotage, ureteroscopy with upper tract biopsies, use of enhanced cystoscopic techniques such as blue light cystoscopy, and/or upper tract imaging [37, 38]. Additionally, random biopsies of the bladder as well as biopsies of the prostatic urethra in men are warranted [39]. Tumor recurrence in patients with non-muscle-invasive bladder cancer occurs in the prosthetic urethra 24–39% of the time [40]. Additionally, upper tract tumors may be discovered in up to 25% of patients with muscle-invasive bladder cancer [41].

Conclusion

In summary, urinary tract cytology is a valuable diagnostic tool in the armamentarium of those treating patients with bladder cancer. Numerous studies have repeatedly demonstrated favorable performance characteristics of urinary tract cytology in the detection of high-grade urothelial carcinoma and carcinoma in situ and poor performance in detecting low-grade urothelial carcinoma. Urologists must be cognizant of the limitations of this study and use discretion when ordering this test for patients. Advances in cytopathologic nomenclature and classification systems such as The Paris System provide a common language for cytopathologists and urologists to more reliably risk-stratify patients. Although urinary tract cytology will not replace cystoscopic evaluation, it will continue to serve as an important adjunctive test to help guide clinical decision-making in intermediate- and high-risk patients.

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Chapter 10
Urinary Biomarkers: Current Status and Future Opportunities

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Introduction

Bladder cancer (BC) consistently ranks in the top ten most expensive cancers to treat and monitor in the United States with estimated cumulative costs of $4 billion annually, which represents 3.2% of all cancer-related care [1]. Cystoscopy is the gold-standard method for both detection and disease surveillance for BC. However, it is invasive and relatively expensive and imposes anxiety, discomfort, and pain on patients. Cystoscopy also carries a risk of infection, hematuria, urethrorrhagia, lower urinary tract symptoms, decreased sexual performance, and decreased quality of life [2, 3]. The procedure itself is not perfect: it is operator-dependent and fails to detect small papillary lesions and up to 30% of flat malignant lesions in the urinary tract when conventional white light cystoscopy is used [4]. For these reasons, urologists have relied on voided cytology as an adjunctive and non-invasive test to supplement cystoscopy in their diagnostic and surveillance decisions for over 50 years. Cytology does have a high sensitivity and specificity for high-grade tumors, but it suffers from high false-positive rates, depending on the experience of the

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interpreter, especially in the settings of previous intravesical therapy, radiation, inflammatory conditions, or neurogenic bladder. The performance of cytology drops considerably for detecting low-grade lesions, and it adds significant diagnostic dilemma when the exfoliated urothelial cells are interpreted as being *atypical*. The high costs of cystoscopy and cytology relative to their limitations for the detection of BC have driven researchers to take advantage of the rapidly evolving field of molecular-based precision medicine to discover urine biomarkers that are highly sensitive, highly specific, and cost-effective across different clinical scenarios.

Multiple urine biomarkers are available commercially for the detection and monitoring of BC, and some have gained Food and Drug Administration (FDA) approval (Tables 10.1 and 10.2). These biomarkers employ different assays to detect nucleic acids, protein antigens, and chromosomal aberrations seen in BC. Nevertheless, the adoption of FDA-approved biomarkers by national and international guidelines is limited because these tests, despite having increased sensitivity over cytology, cannot replace cystoscopy [5–7]. The higher specificity of cytology relative to these biomarkers has also hindered their wide implementation beyond academic research settings.

Cutting-edge, next-generation sequencing and powerful bioinformatic platforms continue to increase our knowledge about the molecular pathways leading to BC genesis, disease progression, and modulation of therapy response. In parallel, numerous novel urine biomarkers are being identified and developed that capture the molecular heterogeneity of BC and thereby increase detection rates. Newer assays are adding DNA mutations, DNA methylation status, regulatory RNA molecules, proteomics, and metabolomics to the pool of BC targets in the test panels, and this has shown great promise in enhancing the diagnostic quality of urine-based tests. This chapter summarizes current commercially available biomarkers for both diagnosis and surveillance of bladder cancer and highlights the scientific rationale of emerging urine biomarkers across the spectrum of protein, genomic, epigenetic, transcriptomic, inflammatory, metabolomic and combination biologic targets (Fig. 10.1).

Defining an Ideal Bladder Cancer Diagnosis and Surveillance Test

In general terms, a biomarker is defined as a marker is of a biological process that can provide information about disease status or future risk of disease [8]. Given the side effects and costs associated with the use of cystoscopy in detection and surveillance for bladder cancer, there is great interest in developing urine-based biomarkers that can outperform voided cytology and either enhance cystoscopy or safely replace it. According to the 2015 World Health Organization/International Consultation on Urological Diseases consensus statement regarding biomarkers for BC detection and surveillance, an ideal biomarker must be *easier*, *better*, *faster*, and

Table 10.1 Summary of commercially available urinary biomarkers for detection of bladder cancer

Biomarker/ manufacturer	FDA approved	Starting material	Target	Assay type	Sensitivity (%)	Specificity (%)	Comments	References
FISH-Urovysion™ Abbott Vysis, USA	Yes	Exfoliated cells	Alterations in chromosomes 3, 7, 17, 9p21	Gene amplification/ aneuploidy detection-FISH	63 (50–75)	87 (79–93)	Interpretation obscured by instrumentation, false-positive rates. Not listed in guidelines as an option for initial diagnosis	[15]
BTA Stat® Polymedco USA	Yes	Protein	Complement factor-H related	Dipstick immunoassay	64 (58–69)	77 (73–81)	POC. Affected by benign conditions	[15]
BTA Trak® Polymedco USA	Yes	Protein	Complement factor-H related	ELISA or colorimetric immunoassay	65 (54–75)	74 (64–82)	Affected by benign conditions	[15]
Cx Bladder Triage™ Pacific Edge NZ	No	mRNA	IGFBP5; HOAX13; MDK; CKD1; CXCR2; age, sex, smoking/hematuria history	RT-qPCR	95	–	Used in hematuria patients with low probability of cancer NPV = 98.5%. In-home sampling available	[25]
Cx Bladder Detect™ Pacific Edge NZ	No	mRNA	IGFBP5; HOAX13; MDK; CKD1; CXCR2	RT-qPCR	82 (70–90) 97(HG)	85 (81–88)	Used in high-risk hematuria patients	[21]
NMP22 BladderChek® Alere USA	Yes	Protein	NMP22	ELISA	64 (58–69)	77 (73–81)	POC. Affected by benign conditions and BP medications	[15]
Xpert Detection® Cepheid USA	No	mRNA	UPK1B; IGF2; CRH; ANXA10; ABL1	RT-qPCR	78 (66–87) 90 (HG)	84 (81–86)	Not affected by benign conditions. NPV = 98%. Turnaround time 90 minutes	[25]

(continued)

Table 10.1 (continued)

Biomarker/ manufacturer	FDA approved	Starting material	Target	Assay type	Sensitivity (%)	Specificity (%)	Comments	References
UroSEEK	No	DNA	TERT; FGFR3; PIK3CA; HRAS; KRAS; TP53; CDKN2A; ERBB2; MLL; MET; VHL	Singleplex PCR (TERT) and multiplex PCR (others)	95 (combined with cytology)	93 (combined with cytology)	Improves detection in atypical cytology samples 96% sensitivity, NPV 99%. Not widely distributed	[27]
Assure MDx™ MDxHealth USA	No	DNA	FGFR3; TERT; HRAS; methylation of OTX1, ONECUT2, TWIST1; age	DNA methylation and PCR	95 (87–98)	85 (79–89)	NPV = 99.6% in adjusted analysis assuming 5–10% prevalence of cancer. May become useful in screening low-risk patients with hematuria	[28]

n/a not applicable, *CIS* carcinoma in situ, *LG* low-grade tumor, *FISH* fluorescence in situ hybridization, *POC* point of care, *CEA* carcinoembryonic antigen, *RT-PCR* reverse-transcription polymerase chain reaction, *q* quantitative, *NPV* negative predictive value, *HG* high-grade tumor, *ELISA* enzyme-linked immunosorbent assay, *BP* blood pressure

Table 10.2 Summary of commercially available urinary biomarkers for surveillance of bladder cancer

Biomarker/ manufacturer	FDA approved	Starting material	Target	Assay type	Sensitivity (%)	Specificity (%)	Comments	References
FISH-Urovysion™ Abbott Vysis, USA	Yes	Exfoliated cells	Alterations in chromosomes 3, 7, 17, 9p21	Gene amplification/ aneuploidy detection-FISH	13–93	65–95	May predict BCG response. Anticipatory positive readings vs. false positives. Expensive	[10]
ImmunoCyt/ uCty+ Scimedx USA	Yes	Exfoliated cells	CEA, 2 mucin-like antigens[a]	Immunofluorescence cytology	75 (64–83)	76 (70–81)	Interpretation highly operator dependent. Weak recommendation by AUA for use in cases of atypical cytology	[15]
BTA Stat® Polymedco USA	Yes	Protein	Complement factor-H related	Dipstick immunoassay	40–72	29–86	POC. Affected by benign conditions	[15]
BTA Trak® Polymedco USA	Yes	Protein	Complement factor-H related	ELISA or colorimetric immunoassay	54–62	68–87	Higher sensitivity for HG than BTA stat	[20]
Cx Bladder Monitor™ Pacific Edge NZ	No	mRNA	IGFBP5; HOAX13; MDK; CKD1; CXCR2; clinical variables[b]	RT-qPCR	92%	–	NPV 96% in patients with atypical cytology. In-home sampling available	[56, 57]
NMP22 BC	Yes	Protein	NMP22	Dipstick immunoassay	61 (49–71)	71 (60–81)	Predictive accuracy of 84.2%[c]	[5, 53]
NMP22 BladderChek	Yes	Protein	NMP22	ELISA	70 (40–89)	83 (75–89)	Accuracy 99% when combined with cystoscopy. May be predictive of recurrence and progression	[5, 46]

(continued)

Table 10.2 (continued)

Biomarker/ manufacturer	FDA approved	Starting material	Target	Assay type	Sensitivity (%)	Specificity (%)	Comments	References
ImmunoCyt/ uCty+ Scimedx USA	Yes	Exfoliated cells	CEA, 2 mucin-like antigens[a]	Immunofluorescence cytology	75 (64–83)	76 (70–81)	Performs better than other FDA-approved biomarkers for LG	[15, 44]
Uromonitor U-monitor PT	No	DNA	TERT; FGFR3	RT-PCR	74	93	100% sensitivity and 89% specificity when combined with cystoscopy. Not affected by benign conditions	[58, 60]
Epicheck Nucleix USA	No	DNA	15 proprietary DNA methylation targets	DNA methylation	68.2 92 (HG)	88	Might be useful in patients with atypical cytology	[61]

[a]Monoclonal antibodies against mucin-like antigens M344 and LDQ10 and a high molecular form of CEA known as 19A211
[b]Clinical variables include primary vs. recurrent tumor, time since previous tumors in years
[c]Based on nomogram including age, gender, cytology, and cutoff of 10 units/milliliter for NMP22

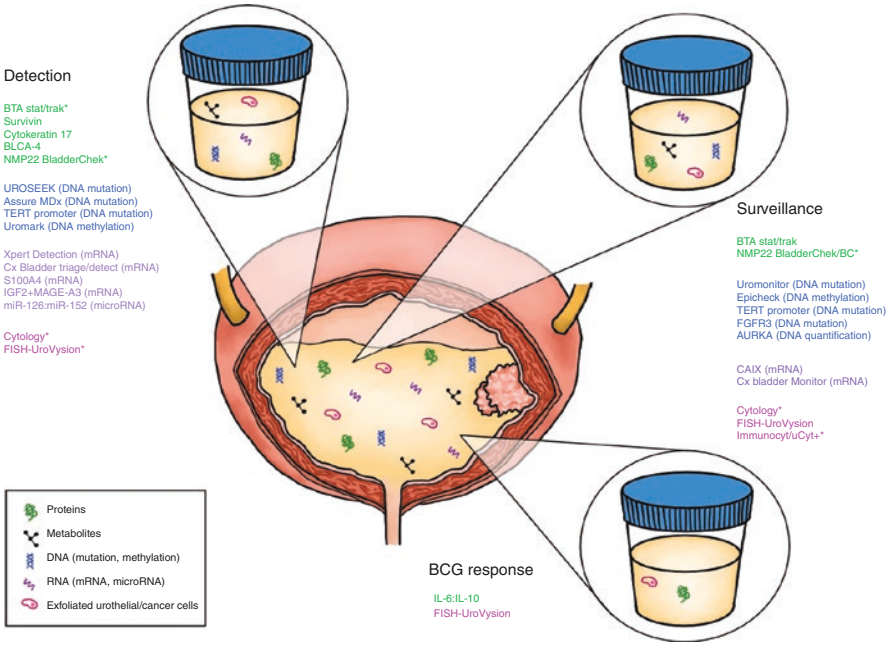

Fig. 10.1 Summary of urinary biomarkers for detection, surveillance, and response to BCG. Selected examples for each class of biomarker (e.g cell-based vs. protein) are included (color-coded). Asterisk denotes FDA-approved markers. FISH fluorescence in situ hybridization, NMP22 nuclear matrix protein, TERT telomerase reverse transcriptase, FGFR3 fibroblast growth factor receptor 3, S100A4 S100 calcium-binding protein A4, IGF2 insulin-like growth factor 2, CAIX carbonic anhydrase IX, AURKA aurora A kinase, BCG bacillus Calmette-Guérin, IL interleukin

cheaper for it to be fully implemented into standard clinical practice [9]. Being *easier* and *better* relates to the ease of sample collection and processing, and the analytical requirements and test reproducibility in different clinical settings (i.e., academic vs. community centers). Being *faster* and *cheaper* relates to the cost-effectiveness of the biomarker taking into account not only the provider and patient's time and convenience (e.g., point-of-care test in the office or at home) but also the financial and quality of life repercussions of false-positive results [10].

The performance of biomarkers is commonly judged by their sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). A threshold of detection for calling a test positive or negative will influence the marker's sensitivity or specificity. In other words, the set threshold determines the likelihood of detecting true vs. false-positive and true vs. false-negative test results. Accordingly, high thresholds will increase specificity while decreasing sensitivity (fewer false positives and more false negatives). Correspondingly, low thresholds will increase sensitivity while decreasing specificity (fewer false negatives and more false positives) [9].

In the clinical setting, providers and patients tend to find predictive values more meaningful and easier to interpret than sensitivity or specificity. PPV defines the probability of the disease in a patient who has a positive test result. It represents the proportion of patients with the disease who have a positive test result in a total group of patients with positive tests results. NPV defines the probability of the absence of the disease in a patient who has a negative result. It is the proportion of healthy patients with negative test results in a total group of patients with negative test results. Predictive values, unlike sensitivity and specificity, are affected by the disease prevalence. This is a concept that has important implications in biomarker development and validation. For instance, the majority of biomarker studies developed for the initial diagnosis of bladder cancer have used case-control cohorts, which have a high prevalence of disease not reflective of actual real-world patterns. This results in an inflated assessment of the biomarker performance, specifically its PPV, which cannot be reproduced in subsequent validation studies. This issue also spills over to the surveillance setting because of the higher prevalence of bladder tumors in this population. The PPV will exceed that of urine cytology and in some instances, the test will be positive before the cystoscopy detects a recurrent tumor. This creates a diagnostic dilemma as there is no easy way to separate false-positive test from true positive tests in the absence of visible tumor [9]. Ultimately, the effective use of biomarkers rests on the providers themselves who need to choose a biomarker based on the performance characteristics that best capture their specific clinical need (e.g., high sensitivity to detect persistent or recurrent high-grade tumor) while also acknowledging the patient's perspective [11].

Current Commercially Available Tests for Bladder Cancer Diagnosis

UroVysion

This is a cell-based test (Abbott Laboratories, Abbott Park, Illinois, USA) that uses fluorescence in situ hybridization (FISH) to detect increased numbers of chromosomes 3, 7, and 17 and deletion of 9p21 (locus for the p16 tumor suppressor gene) in exfoliated urothelial cells present in urine. This test was developed in 2000 and approved by the FDA in 2001 to monitor tumor recurrence in patients with a history of urothelial carcinoma. The label was later extended in 2005 as an aid for initial diagnosis in patients with hematuria suspected of having BC [12]. This assay has one of the highest specificities of the available biomarkers as it is not affected by hematuria, inflammation, and other factors that can lead to false-positive results with other markers. Cumulative data shows that, compared to urine cytology, UroVysion has a higher sensitivity across all grades and stages [13, 14]. A recent meta-analysis showed overall sensitivity of 63% (50–75%) and specificity of 87% (79–93%) [15]. The specificity is overall, lower than cytology for cancer detection.

The two major drawbacks for the use of this test for the detection of bladder cancer are lack of specific criterion to define a cell as being "abnormal" in the context of instrumentation and the false-positive rate, which has been shown to remain significant even after extended follow-up in patients with atypical cytology [16]. In addition, like other markers, UroVysion can perform differently according to patient characteristics (e.g., age and smoking status) and the indication for testing (i.e., presence of symptoms versus cancer surveillance) [17]. It is also possible that not all bladder tumors have the mutations assayed in the current FISH test. In a recent prospective, pathologically confirmed analysis of bladder washed urine specimens, UroVysion did not significantly outperform urine cytology in either sensitivity (67% vs. 69%) or specificity (72% vs. 76%) contradicting pooled studies. Also, 31% of tumors did not have aneuploidy of chromosomes 3, 7, 17, or 9p21 [18]. Currently, UroVysion is not part of national guidelines for initial bladder cancer detection [5].

Bladder Tumor Antigen (BTA)

This assay uses monoclonal antibodies (Polymedco, Cortlandt Manor, New York, USA) to detect complement factor H-related protein and complement factor H, which are found in bladder cancer cell lines that inhibit the complement cascade to prevent cell lysis. The quantitative BTA (BTA Trak^{R}) test is performed in a specialized laboratory (uses colorimetric immunoassay or ELISA), whereas the qualitative BTA (BTA Stat^{R}) is a point-of-care test with an immediate result (dipstick immunoassay). Pooled sensitivity and specificity for BTA Stat^{R} is 64% (58–69%) and 77% (73–81%), respectively [15]. Pooled sensitivity and specificity for the BTA Trak^{R} are 65% (54–75%) and 74% (64–82%), respectively. Both tests, which are FDA-approved for BC detection, have shown higher sensitivity than cytology, but specificity is significantly decreased by benign conditions where the complement factor H-related protein is present including hematuria, nephrolithiasis, inflammation, recent instrumentation, and BCG therapy [19].

Cx Bladder

This assay is a messenger RNA (mRNA)-based test (Pacific Edge Diagnostics, Dunedin, New Zealand) that detects transcripts for five genes: IGFBP5, HOXA13, MDK, CDK1, and CXCR2. This assay was developed as three different tests, including the Triage™, the Detect™, and the Monitor™ for different clinical scenarios. Triage incorporates patient risk profile (age, sex, smoking exposure, hematuria history) to generate a genomic-phenotypic score that can help select patients out of having an unnecessary hematuria workup. In a cohort of 587 patients with gross hematuria, this test showed a sensitivity of 95.1% and a negative predictive value of 98.5%. Forty percent of patients were accurately "triaged out" with a low

probability of cancer [20]. The Detect™ test includes only the quantitative polymerase chain reaction (PCR) panel but is used in high-risk patients with gross hematuria. In a cohort of 485 patients, this assay had an overall sensitivity of 82% (70–90%), which was better than cytology or NMP22 test, and a specificity of 85% (81–88%). The specificity was reduced in patients with history of nephrolithiasis (68%) [21]. The sensitivity for high-grade tumors was 97% suggesting a role for helping in the prioritization of cystoscopies in real-world settings. Medicare currently covers Detect™ and Monitor™. All Cx Bladder tests have not gained FDA approval as of early 2021.

NMP22 BladderChek

Nuclear matrix protein 22 (NMP22) is involved in the distribution of chromatin during mitosis, and it is elevated in malignant urothelial cells compared to normal urothelium [22]. It is released into the urine from apoptotic cells, and the test uses two antibodies against the matrix protein. NMP22 BladderChek is a qualitative point-of-care test (now marketed as the Alere NMP22 BladderChek, Alere, St. Louis, Missouri, USA) that is FDA-approved for both diagnosis in symptomatic patients and follow-up. The test is easy to set up and has a quick turnaround at 30 minutes. Based on 22 studies, BladderChek has a pooled sensitivity and specificity of 64% (58–69%) and specificity of 77% (73–81%), respectively [15]. Like other commercial tests, the sensitivity of this assay is higher than cytology, but it has a significant false-positive rate due to benign conditions and certain medications such as blood pressure-lowering drugs [23]. This assay has shown some utility in screening symptomatic patients with elevated risk for cancer (smoking or other chemical exposures). A study correctly predicted bladder cancer in six cases out of 224 high-risk patients who had elevated NMP22 concentrations for a sensitivity of 97%, specificity of 29%, and NPV of 99% [24]. However, data in support of its use as an initial screening test is still limited.

Xpert Detection

This assay is a mRNA-based test (Cepheid, Sunnyvale, California, USA) that quantifies transcripts for five genes, UPK1B, IGF2, CRH, ANXA10, and ABL1. A multi-institutional study recently validated this assay in a cohort of 828 patients undergoing cystoscopy for microscopic or macroscopic hematuria. Xpert had a sensitivity of 78% (66–87%) overall and 90% (76–96%) for high-grade tumors. The negative predictive value was 98% (97–99%) overall [25]. Combination with cytology did not increase detection rate. This test is not FDA-approved, but it is promising given the improved sensitivity over other tests, its ease of use (does not require a PCR laboratory), and fast turnaround for results (90 minutes). It is also operator independent and seems to not be affected by benign conditions of hematuria.

UroSEEK

This assay uses next-generation sequencing to detect mutations in TERT promoter, which occur in up to 80% of bladder cancers, and 10 additional genes: FGFR3, PIK3CA, HRAS, KRAS, TP53, CDKN2A, ERBB2, MLL, MET, and VHL [26]. In the initial detection cohort, UroSEEK was positive in 83% patients who developed bladder cancer. Combined with cytology, the sensitivity and specificity were 95% and 93%, respectively. The overwhelming majority of tumors investigated showed at least one mutation in the panel. UroSEEK was also effective in detection of cases where the cytology was atypical, predicting the progression of 95% of patients with atypical cytology that developed cancer [27]. A follow-up study with patients with atypical cytology and no previous diagnosis of cancer showed a sensitivity, specificity, and NPV of 96%, 88%, and 99% respectively. Although these results are promising, this assay remains investigational and is not widely distributed yet.

AssureMDx

This is a DNA-based assay (MDx Health, Irvine, California, USA) that detects mutations in FGFR3, TERT, and HRAS in combination with methylation of OTX1, ONECUT2, and TWIST1. These results, in conjunction with patient age, yield a risk profile of BC and may help avoid cystoscopies in patients presenting with hematuria. This assay is still considered investigational and does not have FDA-approval as of early 2021. Pooled sensitivity and specificity from two studies to date are 95% (87–98%) and 85% (79–89%) respectively [28]. The studies adjusted for a prevalence of bladder cancer of 5–10% and showed a negative predictive value of 99.6%, which would reduce 77% of their cystoscopies [29, 30]. This test might be useful for screening a low-risk patient with symptomatic hematuria and awaits further prospective validation from an ongoing clinical trial in the United States (NCT03122964).

Current Commercial Tests for Bladder Cancer Surveillance

UroVysion

UroVysion has been shown helpful in defining patients at higher risk of recurrence who have an atypical or negative cytology. “Anticipatory positive” readings are thought to reflect chromosomal changes before the development of phenotypic expression of cancer and therefore are not considered to be false positives. Several reports have shown a large proportion of patients with positive readings, but negative cystoscopies will eventually have clinically detectable tumors within 2 years

[31–33]. Moreover, patients with negative FISH are unlikely to experience recurrence in less than 1 year [32]. Studies overall show better specificity when this test is used for high-grade bladder cancer surveillance especially in carcinoma in situ [14, 34, 35]. There is also data supporting a role of Urovysion in predicting response to BCG. Having a positive test post-BCG increased the risk for tumor recurrence three to five times higher than those patients with a negative test [36–39]. There is also data suggesting that positive Urovysion may predict a higher likelihood of progression while on BCG [40, 41]. Based on this, this test has been recently proposed as a form to detect "molecular BCG failure" in patients with negative surveillance cystoscopies but positive FISH soon after BCG induction [42]. However, current national guidelines do not have a strong recommendation for the routine use of UroVysion in cancer surveillance aside from helping to interpret indeterminate cytology [6, 5]. This test remains limited to assisting urologists in patient counseling and recruitment into clinical trials comparing BCG to novel intravesical and systemic agents [43].

ImmunoCyt/uCyt+

This is a cell-based test (Scimedx, Denville, New Jersey, USA) that combines cytology and an immunofluorescence assay. It uses fluorescent-labeled monoclonal antibodies against a glycosylated form of carcinoembryonic antigen and two bladder mucins. These antigens on exfoliated urothelial cells are specific for bladder cancer. The assay is not affected by benign conditions, but it requires a trained cytopathologist and a minimum of 500 cells in the sample for validity. It was first introduced in 1997 and is FDA-approved for surveillance only. This test performs better than other FDA-approved biomarkers in the detection of low-grade tumors [44]. In a recent metanalysis combining eight studies, the sensitivity and specificity were 75% (64–83%) and 76% (70–81%), respectively [15]. One prospective study of 91 patients demonstrated no association between this marker and recurrence-free survival or progression-free survival [45]. In patients with a negative cytology but positive test, uCyt+ was not predictive of recurrence [46]. Diffusion of this test remains limited given that interpretation is complex and highly operator-dependent. AUA has a weak recommendation for its use in patients with atypical cytology.

BTA Stat/BTA Trak

The sensitivity, specificity, and negative predictive value of BTA stat range between 40% and 72%, 29% and 86%, and 38% and 77%, respectively [22]. One recent prospective study comparing this marker to other commercially available markers showed that BTA stat was not predictive of recurrence or progression [45]. In a cohort of 368 patients with a positive test but initial negative cystoscopy, the risk of

undetected recurrence was about 16% after additional investigation with imaging and random biopsies [47]. Studies for BTA trak are more limited. Overall, the sensitivity and specificity range from 54% to 62% and 68% to 87% respectively [22]. One study did report an 88.4% negative predictive value [48]. Some studies did compare BTA trak to BTA stat, and the performance between the tests was similar although BTA trak had a higher sensitivity for detecting recurrent tumors [49]. These tests gained their FDA approval as adjuncts to cystoscopy for surveillance purpose (not detection), but their clinical use remains limited due to the high false-positive rates (up to 80%) in patients with hematuria and/or benign conditions [50, 51].

NMP22 BladderChek/NMP22-BC

BladderCheck is also FDA-approved for surveillance. Pooled sensitivity and specificity are 70% (40–89%) and 83% (75–89%), respectively [5]. A multicenter study of 668 patients on surveillance showed that combining cystoscopy with BladderChek resulted in an increased diagnostic accuracy to 99% (94–100%), which was better than cystoscopy alone [52]. One study comparing BladderChek to BTA Stat and Immunocyt/uCyt showed that BladderChek was the only marker predictive of recurrence and progression [46]. NMP22-BC is the quantitative counterpart to BladderChek. This test is an ELISA test which utilizes two monoclonal antibodies. Currently, this test is FDA-approved for surveillance only. Like BladderChek, NMP22-BC test has higher sensitivity than cytology but lower specificity. Compared to BladderChek, the sensitivity and specificity for this test are lower at 61% (49–71%) and 71% (60–81%), respectively [5]. A study generated a nomogram including age, gender, and cytology, and NMP22 using the manufacturer's cutoff point of 10 units/milliliter [53] showed a predictive accuracy of any recurrence at 84.2%. Overall, NMP22 tests can be used as an adjunctive tool for the detection of bladder cancer, but diagnostic performance for surveillance is limited when used alone or in even in combination with cytology [54].

Cx Bladder Monitor

This assay was analyzed in a prospective study of 763 patients with prior non-muscle-invasive disease and added previous tumor occurrence information (primary vs. recurrent tumor, time since previous tumors in years) to the five gene expression panel measured in Cx bladder triage™ and detect™. The sensitivity and negative predictive value were 92% and 96%, respectively. The performance was independent of stage, grade, and BCG treatment regardless of interval since treatment completion [55]. A follow-up study compared this assay to FDA-approved assays including NMP22 ELISA, NMP22 BladderChek, and cytology in 803 patients. Cx

bladder had better sensitivity and NPV than all tests including cytology at 91% and 96%, respectively [56]. In a pooled retrospective analysis of 852 patients with atypical cytology with or without an equivocal cystoscopy, this assay had an NPV value of 97% (94–98%), which would avoid unnecessary cystoscopies in 35% of patients [57]. A retrospective audit of cystoscopy patterns in New Zealand after the incorporation of Cx bladder monitor™ into a real-world surveillance cohort showed that this test was useful in identifying patients with lower risk of recurrence; recurrence risk was 16.2-fold lower in patients with a negative test than a positive test. About 77.8% of patients were safely managed with one cystoscopy per year without compromising detection rates, thereby reducing the overall cystoscopy burden. Results so far are very promising, although all three Cx bladder tests are not currently FDA-approved as more prospective validation is needed.

Uromonitor

This test is an RT PCR assay (U-Monitor, Porto, Portugal) optimized for the detection of hotspots mutations in TERT promoter (telomerase reverse transcriptase) and FGFR3 (fibroblast growth factor receptor 3) genes in DNA from exfoliated urothelial cells. The test is processed by a central laboratory and turnaround is 3–5 days. It was first validated in 331 urine samples, and it showed a sensitivity of 74% and specificity of 93% in detecting recurrence, which were comparable to cystoscopy alone. When combined with cystoscopy, this test achieved 100% sensitivity and 89% specificity, which was higher than the sensitivity and specificity of cystoscopy combined with cytology (sensitivity of 87% and specificity of 88%) [58]. Recently, mutations in the oncogene KRAS were added to the panel (Uromonitor-V2), and this was tested in 97 patients undergoing surveillance for non-muscle-invasive disease. The sensitivity was improved to 93.1% and the NPV was 95%, which was higher than for cytology making it a promising alternative to cytology/cystoscopy [59]. Moreover, the presence of inflammation or other benign lesions does not seem to affect the performance of this test [60].

Epicheck

This assay includes 15 proprietary DNA methylation markers (Nucleix, San Diego, California, USA) commonly altered in bladder cancer. DNA is extracted from the cell pellet of centrifuged urine and digested with a methylation-sensitive restriction enzyme. The assay then uses reverse transcription (RT) PCR with locus-specific primers. The report for each patient contains a quantitative score (EpiScore) and a positive/negative interpretation. The EpiScore is a number between 0 and 100, with a higher score indicating more methylation; an EpiScore $\geq$60 is considered a positive result [61]. The original report in 353 showed an overall sensitivity and

specificity of 68.2% and 88%, respectively. Excluding low-grade disease, the sensitivity and NPV improved to 92% and 99%, respectively. Age, gender, treatment for recent recurrence, smoking history, and occupational exposure had no impact on the test performance [61]. A follow-up study tested this assay against cytology and showed a higher sensitivity (62% vs. 33%) especially for high-grade tumors (83% vs. 46%). Specificity was however higher for cytology at 98.6% % vs. EpiCheck at 86.3% [62]. At this time, this assay cannot replace cystoscopy or cytology based on this data alone. Nevertheless, Epicheck may have the potential to reduce surveillance cystoscopies in patients with either atypical or suspicious for high-grade carcinoma cytological interpretations [63]. Given its cost and technical challenges compared to other commercial assays, the implementation of Epicheck in surveillance practices remains limited.

Potential Urinary Biomarkers

Protein Biomarkers

Protein-based biomarkers have been heavily studied for the detection of bladder cancer and are quantified by either immunoassays or spectrometry. Testing urine protein biomarkers is convenient as they lend themselves to the creation of efficient point-of-care tests that yield fast results as opposed to more complex molecular techniques, which require optimal sample collection and can only be performed by specialized laboratories. One of the most widely studied proteins is survivin, which has been shown to promote cell proliferation and enhance angiogenesis in a variety of tumors [22]. Quantification of survivin using ELISA reports a sensitivity of 71–85% with a specificity of 81–95% for the detection of BC [64]. Survivin has been shown to be associated with both disease recurrence and disease-specific mortality [65]. Orosomucoid 1 (ORM1) modulates the activity of the immune system during the acute-phase reaction. It was found to be significantly elevated in bladder cancer patients compared to controls or those with benign conditions and had a sensitivity, specificity, and AUC of 92%, 94%, and 0.965, respectively [66]. The serine protease HtrA1 which has been implicated in multiple cancers achieved a sensitivity of 93%, specificity of 96%, and AUC of 0.98. Two isoforms of this protein were detectable in urine, and it was significantly downexpressed in cancer patients compared to both healthy and patients with cystitis. Keratin 17 (K17) is a member of the cytokeratin family, which has been shown to facilitate escape from G1-S phase cell cycle control, thus leading to cell proliferation in cancer, showed a sensitivity of 100% and specificity of 96% in the detection of BC. Interestingly, immunohistochemistry of biopsy and cytology samples detected K17 in low- and high-grade lesions but not in normal urothelium suggesting a role for this biomarker in monitoring disease recurrence as an adjunct to cystoscopy and cytology [67].

Additional individual protein markers include APO-A1 (apolipoprotein-A1), BLCA-4, and hyaluronidase, which have been independently validated by two or more studies in the detection setting and overall surpass the sensitivities (89–95%, 93%, 89–100%) and specificities (85–92%, 97%, 89–91%) of that of FDA-approved NMP22 and BTA protein-based assays [68]. Panels measuring multiple proteins have improved assay specificity. For example, a five-panel biomarker using gamma synuclein with Coronin-1A, APO-A4, Semenogelin-2, and DJ-1/PARK7 achieved a sensitivity, specificity, and AUC of 79%, 100%, and 0.92, respectively. Performance was not affected by hematuria or pyuria [69]. There are fewer studies analyzing the performance of protein biomarkers specific for surveillance or response to treatment, but data is very promising for combination panels. For instance, a multiparameter including six biomarkers (cadherin-1, IL-8, ErbB2, IL-6, EN2, and VEGF-A) and three clinical parameters (number of past recurrences, number of BCG therapies, and stage at the time of diagnosis) yielded an AUC of 0.92 for the detection of recurrence outperforming the individual biomarkers [70]. Larger independent validation is required for most combination panels.

Genomic Biomarkers

Telomerase

Hotspot mutations in the telomerase reverse transcriptase promoter (TERTp) are present in >70% of bladder cancers making it the most common genomic aberration independent of stage or grade [58]. For primary diagnosis, the sensitivity and specificity of PCR-based assays range from 62–87% and 84–100%, respectively [71–75]. A recent nested case-control study showed that TERTp mutations were detectable in urine up to 10 years prior to diagnosis at a sensitivity of 47% and an estimated NPV of 99.95% [76]. TERTp mutations are also associated with a five-fold increase in relative risk of recurrence [73]. One of the main advantages of this biomarker is that mutations in adjacent normal urothelium are present at very low frequencies and are not typically detected in inflammatory (e.g., post BCG) or infectious disease states. This makes it a useful marker in the surveillance period although studies with larger cohorts are needed to justify replacing the use of cytology with this biomarker [77]. TERTp mutations are currently included in commercial tests such as Uromonitor, AssureMDx, and the UroSEEK assay.

Fibroblast Growth Factor Receptor 3

Mutations in FGFR3 are detected in approximately half of bladder cancer patients and in about 60–70% of low-grade tumors [78]. As a standalone test, FGFR3 achieves a sensitivity of 39% for the initial detection of bladder cancer [79]. In the surveillance period, the presence of FGFR3 mutations in the urine was shown to be predictive of recurrence with a sensitivity of 70% and specificity of 87%. The time

to recurrence was significantly shorter for patients with positive assays vs. those with only negative assays [79]. In a cohort of 200 patients with superficial low-grade tumors, the sensitivity for the detection of concomitant recurrence was 58%, while a positive test was associated with a fourfold higher risk of recurrence during follow-up [80]. FGFR3 mutations may be helpful in identifying patients with a good prognosis, and low risk of progression as FGFR3 mutant incident tumors tend to be of lower stage and grade than recurrent tumors in patients with FGFR3 wild-type incident tumors [81]. Along these lines, a modified surveillance protocol with partial replacement of cystoscopy based on FGFR3 mutation status was found to be safe and more cost-effective than standard surveillance in a decision analytical study (Markov model) of 70 patients. Patients mostly had Ta/Grade 1 tumors and had a median follow-up of 8.8 years [82].

Aurora Kinase A

Aurora kinase A (AURKA) is a serine/threonine protein kinase that plays an important role in mitosis and is overexpressed in several cancers including BC [83]. AURKA overexpression has been associated with poor clinical outcomes and attributed to increased cell cycle progression and genomic instability with aneuploidy [83]. AURKA may also predict resistance to neoadjuvant cisplatin-based chemotherapy in patients with muscle-invasive BC [84]. In addition, AURKA may regulate cell invasion and metastasis. A recent analysis of 423 bladder cancers using tissue microarray showed that overexpression of AURKA combined with expression signature of AURKA downstream regulatory genes (e.g., transcription factor PAX-3) was enriched in basal subtype tumors, which were highly aggressive [85]. AURKA can be measured in voided urine via a FISH or RT-PCR assay. The FISH assay measures AURKA copy number in urothelial cells and has a sensitivity and specificity for bladder cancer detection that ranges from 80% to 97% and from 80% to 87%, respectively [86, 85]. One study using RT-PCR in voided samples of patients with hematuria showed a sensitivity of 84% and specificity of 65%, respectively. Compared to cytology, the accuracy of this biomarker was higher for the detection of low-grade lesions [87]. One study compared FISH for AURKA to UroVysion and showed it was equally effective and less expensive for the detection and monitoring of BC [88]. More prospective studies are needed to support the use of AURKA as a biomarker for the detection and surveillance of bladder cancer, and as it stands, it cannot completely substitute cytology.

Epigenetic Biomarkers

DNA Methylation

DNA methylation alterations associated with aging and the cumulative effects of environmental exposures play an important role in BC carcinogenesis and progression. Hypermethylation of GC-rich regions within a gene promoter known as

CpG islands results in silencing of tumor suppressor genes [89]. Alternatively, global loss of DNA methylation (hypomethylation) results in aberrant gene expression. DNA methylation changes are chemically stable and can be quantified from cell-free DNA fragments and tumor cells shed in urine [90]. The first study demonstrating the feasibility of urine-based DNA methylation assays for BC diagnosis showed a sensitivity of 91% and specificity of 76% for a panel including RARß, DAPK, E-cadherin, and p16 [91]. Subsequently, over one hundred more methylation-based markers have been reported across numerous studies [92]. The trend has been to improve the sensitivity and specificity of methylation based-assays relative to cytology by continuing to test different combinations rather than individual ones. In the detection setting, the sensitivity and specificity of panels including combination markers have ranged from 52% to 100% and from 60% to 100%, respectively [89, 90]. The combination of POU4F2 and PCDH17 showed a sensitivity and specificity of 90% and 94% respectively with an area under the receiver operating characteristics curve (AUC) of 0.92. A prospective multicenter study using patients with benign urologic diseases as controls showed a sensitivity of 90% and specificity of 93% for the combination of NID2 and TWIST1 genes. Interestingly, two follow-up prospective studies evaluating these two genes failed to replicate the high diagnostic performance for detection observed in the original study with sensitivities and specificities of 58–75% and 61–71%, respectively [93, 94]. In addition, prior BCG treatment seemed to affect the accuracy of the panel, while sensitivity was improved in patients categorized as current smokers [94]. The UroMark assay was recently developed for the primary detection of bladder cancer in patients with hematuria. Targeted bisulfite sequencing was used to develop a methylation signature of 150 CpG loci covering a wide spectrum of grades and stages. This assay was validated in two independent sets, and it showed a sensitivity of 98%, a specificity of 97%, and an AUC of 0.97 [95]. Two prospective observational studies DETECT I (NCT02676180) and DETECT II (NCT02781428) are currently underway to validate the use of this assay in both the detection and surveillance settings. Data in support of the use of DNA methylation markers in the surveillance setting is also promising. One prospective study with up to 7.4 years of follow-up showed that a combination of SOX1, IRAK, and L1-MET yielded a sensitivity of 80%, specificity of 97%, and an AUC of 0.90 for detecting disease recurrence [96]. A panel containing CFTR, SALL3, and TWIST1 in combination with cytology showed improved sensitivity for the detection of recurrent tumors (97%). Performing a cystoscopy only in patients with a positive panel would have resulted in an estimated 36% reduction of unnecessary cystoscopies without missing high-grade tumors [97]. Overall, there is a lack of both comparative trials between DNA methylation biomarkers and external validation with larger-scale prospective studies. Differences in techniques (e.g., pyrosequencing vs. quantitative methylation PCR) have also led to significant variability in performance characteristics of methylation-based biomarkers, hampering their adoption into the clinical settings.

MicroRNAs

MicroRNAs (miRNA) are small single-stranded noncoding RNAs (containing about 22 nucleotides) that affect the transcription of genes via base-pairing with complementary sequences within mRNA molecules. This interaction leads to suppression of translation through the interference of complex formation or mRNA degradation (similarly to small interfering RNAs) [98]. miRNAs can be detected in urine as free circulating molecules, bound to ribonucleoprotein complexes, or in extracellular vesicles such as exosomes [99]. These molecules are attractive targets for detection and prognosis in BC as changes in miRNA expression in cancer exhibit tissue specificity with a high level of detectability [90]. Additionally, because of their short length, they are more resistant to degradation by nucleases when compared to other nucleic acids. This simplifies the initial processing of samples as a material can be stored up to 48 hours at room temperature [100]. Targets are detected by miRNA arrays or next-generation sequencing and then quantified by RT-PCR to indicate increased or decreased expression relative to non-cancer controls. Multiple miRNAs have been identified as promising urine biomarkers. Sensitivity and specificity improve when the analysis includes multiple targets instead of single ones. One of the earliest studies showed that miR-126:miR-152 and miR-182:miR-152 ratios were significantly higher in the urine of BC patients compared with healthy donors. The miR-126:miR-152 miRNA ratio yielded the highest sensitivity at 72% and specificity at 82% with an AUC of 0.77 [101]. Another study performed a global miRNA expression profiling analysis and identified a six-miRNA diagnostic signature with a sensitivity of 85% and a specificity of 87% and an AUC of 0.92 for initial diagnosis (miR-18a, miR-25, miR-187, miR-140-5p, miR-142-3p, miR-204) [102]. A similar study identified a 25-miRNA prediction model for diagnosis with estimated sensitivity, specificity, and AUC of 87%, 100%, and 0.92, respectively. Limiting the model to 15 and 10 miRNAs resulted in some loss of performance but revealed miRNAs (miR-140-5p, miR-199a-3p, miR-93, miR-652, miR-1305, miR-224, miR-96, miR-766) that consistently contributed to all models [98]. Some miRNAs have also been proven useful in discriminating high- from low-grade tumors with a sensitivity, a specificity, and an AUC of 81–85%, 74–87%, and 83%, respectively, using miR-125b alone or in combination with miR-92a [102, 103]. Data supporting the use of miRNA during surveillance is less robust. A small study using a panel of 12 miRNAs showed high sensitivity (88%) but low specificity (48%) in an independently validated cohort undergoing surveillance. The performance however was highest for T1 stage (AUC = 0.93) and high-volume disease (AUC = 0.81). More validating prospective studies are needed in this setting. In addition, standardization of urine collection is important as it has been shown to affect the overall performance of miRNA panels. The highest sensitivity was observed in studies using cell-free DNA from urine supernatant rather than whole-urine or urine sediment [100]. This is particularly relevant in settings of hematuria where miRNAs may be released from lyzed erythrocytes resulting in

interference and inaccurate measurement of dysregulated miRNAs in bladder tissue [99].

Transcriptomic Biomarkers

Measurements of mRNA using RT-PCR combined with several RNA amplification techniques (e.g., gold nanoparticles) makes it possible to detect mRNAs in urine despite being mostly degraded by RNases. mRNAs are attractive biomarkers as they reflect active intracellular processes that are different in BC patients when compared to healthy individuals. For example, urine ubiquitin-conjugating enzyme E2C (UBE2C) and isoleucine glutamine motif-containing GTPase-activating proteins (IQGAP3) mRNA levels are significantly higher in BC patients than in healthy controls including those with hematuria [104]. The performance of mRNA tests varies widely across individual and multiple target panels. Notably, multiple targets or the combination of individual targets with cytology has been shown to improve performance in the primary detection setting. Overall, multi-target panels have a sensitivity of 36–97%, a specificity of 82–100%, and an AUC of 0.86–0.95 [64]. S100 calcium-binding protein A4 (S100A4), which encodes a protein that stimulates angiogenesis, was shown to have a sensitivity and specificity of 90% and 92%, respectively, with an AUC of 0.978. Positive rates were higher with increasing stage and grade, and combination with cytology increased the sensitivity of this biomarker to 97% but reduced its specificity to 80% [105]. Carbonic anhydrase IX (CAIX) is a highly expressed gene in cancer cells), and it facilitates cell adaptation to acidic conditions within the tumor microenvironment [106]. It was validated in an independent cohort for detection of tumors achieving a sensitivity and specificity of 81% and 96%, respectively. The predictive accuracy was also significantly higher than cytology, specifically in low-grade tumors (88.3% vs. 67.4%). Interestingly, mRNA levels of CAIX decreased with increasing tumor stage and grade in contrast to what was observed for S100A4. CAIX was also shown to be associated with greater risk of disease recurrence, although its utility for prognosis in the surveillance setting has not been validated [107]. A gene expression signature including insulin-like growth factor 2 (IGF2) and MAGE-A3 has been externally validated in a prospective, blinded, multicenter study for the purposes of detection. The test reached a sensitivity of 81%, a specificity of 91%, and an AUC of 0.94. MAGE-A3 is a tumor-specific protein shown to be expressed in 43% of bladder cancers [108]. More recent studies have focused on developing multi-mRNA signatures to help with risk-stratification and prognosis. Notably, a 13-mRNA signature including proto-oncogene forkhead box M1 (FOXM1), S100 calcium-binding protein A8 (S100A8), and additional genes implicated in increased stage and decreased survival outperformed previously published mRNA signatures. It reached an AUC of 0.90 for the prediction of disease progression with the inclusion of age and grade in the nomogram. Similar to other nucleic-acid-based tests, studies evaluating novel mRNA-based biomarkers currently lack external validation and suffer from

variability in assay development and control group selection, which limits their reproducibility and implementation into real-world settings.

Inflammatory Biomarkers

Host immune response has an impact on tumor development, treatment effect, and tumor progression. For this reason, there has been great interest in identifying disease-specific inflammatory biomarkers that can aid in both diagnosis and post intravesical treatment surveillance. Dysregulated expressions of several cytokines in urothelium as detected in urine and/or blood have been linked to both cancer initiation and progression. These include increased expression and gene polymorphisms in the gene promoter of TNF-alpha; increased expression of TGF-beta; increased expression of interleukin (IL) 1B, IL-6, IL-8, IL-10, IL-13, and IL-18; and decreased expression of IL-16 [109]. In a study including patients with hematuria, IL-13 and heat-shock protein 60 (a protein chaperone) together generated an AUC of 0.95 for the detection of bladder cancer. Inflammatory biomarkers have shown the most promise in the prediction of BCG response and recurrence. For example, a ratio of IL-6/IL-10 predicted BCG response and recurrence-free survival in high-risk patients although CIS patients were excluded [110]. Notably, a prospective study serially measured cytokines in the urine of patients undergoing BCG treatment and constructed a nomogram based on nine cytokines (IL-2, IL-8, IL-6, IL-1ra, IL-10, IL-12(p70), IL-12(p40), TRAIL, and TNF-alpha), which predicted the likelihood of recurrence with 85.5% accuracy (95% CI 77.9–93.1%) [111]. While these studies currently lack external validation, they highlight the increasing importance of inflammatory biomarkers. In the current era of immune checkpoint inhibition and other novel immunotherapies that are changing the treatment paradigm for non-muscle-invasive BC, the study and use of inflammatory markers are increasingly relevant.

Metabolic Biomarkers

Metabolomic signatures can be derived from urine using liquid chromatography and mass spectrophotometry. This is attractive for the development of biomarkers for bladder cancer detection given both the noninvasive nature of sample collection and the ease of sample processing. Metabolic signatures are cancer-specific and can be discerned from metabolic signatures seen in benign hematuria [112]. Thus far, metabolomic studies have remained focused on initial cancer detection. Overall performance has been relatively high with an AUC ranging from 0.81 to 0.99 for panels including three or more metabolites [64, 113]. Sex and age can affect inter-individual variations in the metabolites detected in urine, so incorporation of these factors is important in discovery cohort selection [112]. One study recently compared

metabolic signatures in low- and high-grade bladder cancer with or without hematuria using a gender- and age-matched cohort. In the external validation set, the metabolic panel had a sensitivity, a specificity, and an AUC of 81%, 82%, and 0.84, respectively, for discriminating cancer patients from controls. The AUC for discriminating low-grade vs. control group was 0.90 when selecting the five metabolites with the highest performances out of a panel of 74. The performance for discriminating low- vs. high-grade disease was lower overall with an AUC of 0.83 in patients with hematuria and 0.76 in patients without hematuria [114]. Data on the use of metabolic signatures for surveillance and prognosis is limited. Interestingly, a recent small study measured the changes in the metabolic profile of patients before and after TURBT and noted a statistically significant shift in the profile before and after TURBT. Longitudinal analysis subsequently revealed a gradual shift towards the profile present before TURBT, and in one case, it anticipated the results obtained by cystoscopy thereby supporting the clinical use of metabolic profiling in the surveillance setting [115]. The overall challenge in the validation and future implementation of metabolic markers lies in the inter- and intraindividual measured variability that results from confounding factors such as water intake, diet, drug intake, and environmental exposures.

Combination Biomarkers

Biomarkers across multiple *-omics* categories are now being identified secondary to advances in next-generation sequencing, computational biology, and machine learning. This reflects the increasing appreciation for the significant tumor heterogeneity seen in BC and has driven researchers to integrate *multi-omic* biomolecules in test panels to increase the accuracy of diagnosis and prognostic predictions of patients with cancer [116, 117]. This approach is the basis for commercial tests such as AssureMDx, with additional novel biomarkers published in the last 10 years demonstrating sensitivities and specificities of 90% or greater for detection [64]. One prospective blinded study of 475 patients with gross hematuria incorporated a panel of mutations in TERT and FGFR3 combined with methylation in SALL3, ONECUT2, CCNA1, BCL2, EOMES, and VIM. The sensitivity, specificity, and AUC were 97%, 77%, and 0.96, respectively. The NPV was 99% suggesting a role for this test in triaging patients appropriate for cystoscopy. Of note, 3 out of 99 patients who had a false-negative DNA test in both pre- and post-cystoscopy urine samples were negative for all biomarkers in their tumors [72]. A published a series of reports on individual panels combining cytology with different protein and transcriptomics targets (survivin, hyaluronidase, and matrix metalloproteinase 2 and 9) achieved sensitivities of 83–95% and specificities of 83–98% [118, 119]. A protein-epigenetic combination of HYAL1 (protein), long noncoding RNA-urothelial cancer associated 1 (mRNA), miR-210, and miR-96 showed a sensitivity of 100%, a specificity of 89%, and an ROC of 0.98 [120]. These studies were case-control studies and have not yet been externally validated.

Conclusions

Thanks to advances in next-generation sequencing, proteomics, bioinformatics, and mathematical modeling, significant progress has been made in the development of novel urine biomarkers for the detection and surveillance of BC. Several cell- and protein-based tests are commercially available; however, they remain to be fully endorsed by national and international guidelines due to clinician skepticism in the setting of elevated false-positive rates and heterogeneity present in most studies. Overall, commercial tests have a higher sensitivity but lower specificity than cytology and for this reason cannot be used as standalone tests. Newer markers have shown high performance in both detection and surveillance surpassing that of cytology, yet the vast majority lack external validation in prospective studies. Head-to-head studies are also virtually nonexistent limiting their widespread beyond research settings. Additional unresolved issues include the lack of standardization in sample processing and laboratory methodology (cell-free DNA vs. sediment DNA, sequencing pipelines, etc) and variation in determining thresholds of detection across heterogeneous populations. The foremost challenge for clinicians is the selection of the "best" biomarker for the clinical scenario at hand while understanding potential confounding patient variables (e.g., chronic inflammation) and risk. Nevertheless, there is great potential for improving test performance by capturing the heterogeneity of urothelial tumors through combination biomarkers, which will in turn allow for a personalized approach across multiple care settings.

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Chapter 11
Bladder Cancer Genomics: Indications for Sequencing and Diagnostic Implications

Andrew T. Lenis and Eugene J. Pietzak

Introduction

Despite being a common cancer with a diverse and often unpredictable clinical course, bladder cancer diagnosis and management are still largely based on histologic assessment without tumor genomic profiling or routine molecular characterization. By contrast, in other malignancies, assessment for alterations known to have clinical impact on prognosis or treatment selection is guideline-recommended. For example, the National Comprehensive Cancer Network (NCCN) recommends that patients with non-small cell lung cancer undergo a panel of molecular tests to evaluate for the presence of alterations that are known to affect clinical outcomes [1]. The potential benefits of molecular characterization of malignancy include improved ability to convey prognosis to patients and their families, identify biomarkers predictive of treatment response, and identify actionable alterations for therapies, among others.

Over the last 10–15 years, there has been an influx of data that has advanced our understanding of the molecular biology of bladder cancer and has highlighted the potential utility of genomic sequencing for the diagnosis and management of patients with this disease. Bladder cancer is known to carry a significant mutational burden, akin to lung cancer and melanoma [2]. Genomic sequencing of tumors reveals a rich landscape of alterations. Some alterations are shared across grades and stages of the disease which suggest early events in tumorigenesis, while others are unique and provide insights into underlying disease biology. Further, there is significant interest in genomic sequencing to identify both prognostic and predictive

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biomarkers to the increasing armamentarium of local and systemic treatments for bladder cancer.

In this chapter, we review the literature supporting the role of genomic sequencing for the diagnosis and treatment of patients with bladder cancer. We first provide a primer on next-generation sequencing (NGS), including key concepts and limitations. We then review the data on sequencing for bladder cancer stratified by stage (i.e., muscle-invasive, non-muscle-invasive, and advanced/metastatic bladder cancer). We highlight the increasingly recognized importance of germline testing and address advanced approaches, such as liquid biopsy, that have the potential to radically change management in bladder cancer, especially in the adjuvant setting. Finally, we review the current guidelines and provide practical considerations in using genomic sequencing in the management of patients with bladder cancer.

Primer on Next-Generation Sequencing

Next-generation sequencing (NGS) refers to the process that reads the order of nucleic acids in DNA or RNA [3]. NGS is a significant improvement over prior methods (i.e., Sanger sequencing) in that reactions and analyses can be performed simultaneously, decreasing the time and cost of sequencing. Briefly, in DNA sequencing, the genetic material is first extracted, and a library is generated by fragmenting the DNA and adding specific adaptors. Sequencing can span from whole genome sequencing (WGS) and whole exome sequencing (WES) to more targeted panels consisting of a variable number of genes that are specific to certain pathologies or pathways. Sequencing can be performed on DNA from tumor tissue to identify somatic mutations or on normal tissues (e.g., white blood cells in a plasma sample or histologically normal tissue in a surgical pathology specimen) to identify germline mutations. The number of times a specific nucleotide is sequenced is called the coverage depth [4]. Unlike sequencing of normal tissue for germline alterations, the coverage depth required for somatic tissue sequencing is significantly greater in order to overcome contamination of the specimen by benign tissue (e.g., stromal components). Typical coverage for somatic sequencing is on the order of 1000× compared with germline sequencing coverage of around 30×. Further, mutations present in subclonal populations require higher coverage depth to detect these alterations with lower variant allele frequencies (VAF).

An important consideration in NGS is how the normal sample is derived to which the somatic tumor sequencing is compared [5]. Some assays use patient-matched normal samples, such as morphologically normal tissue like a benign lymph node in a radical cystectomy specimen or the white blood cells in a blood sample [6]. This strategy, compared with a reference genome, reduces the rates of false-positive somatic mutational calls from either germline mutations or clonal hematopoiesis of indeterminate potential.

NGS identifies multiple types of alterations, such as single nucleotide variants (SNVs), as well as structural changes (insertions and deletions) and chromosomal

rearrangements (translocations, duplications, and deletions). Tumor mutational burden (TMB) and microsatellite instability (MSI), both of which may correlate with response to treatment with immunotherapy, can also be derived from NGS data. These alterations can be prognostic and/or predictive of response to treatment. Some alterations are targetable with various therapies. Collectively, alterations that are predictive, prognostic, and/or targetable are considered clinically actionable. Publicly available databases, such as OncoKB, provide curated information regarding the actionability of various alterations in many different cancer types based on guidelines, active clinical trials, and published scientific literature [7].

While NGS has led to innumerable advances in oncology and urologic oncology, several limitations are notable and important to consider. First, known intra-tumoral heterogeneity in primary bladder tumors can result in missed mutations depending on the area sequenced. There is also the issue of depth of coverage for alterations of low VAF. Second, tumor sequencing is generally performed on the primary tumor; however, differences between the primary tumor and the metastases (i.e., inter-tumoral heterogeneity) are known to exist. These differences are the result of clonal evolution and may be promoted by intervening treatments [8]. The clinical implications of these discrepancies between the primary tumor and metastases are not yet fully understood. Future studies are required to determine when and which tissues should be sequenced to best inform treatment decisions and optimize clinical outcomes.

Muscle-Invasive Bladder Cancer

Muscle-invasive bladder cancer (MIBC) is the disease state most fully characterized by genomic sequencing. Efforts have focused on the actionable genomic landscape, driver alterations in divergent differentiation, and biomarkers of treatment sensitivity.

Molecular characterization of bladder cancer was launched by the publication of two seminal manuscripts from The Cancer Genome Atlas (TCGA) [9, 10]. The first publication reported on 131 tumors, and the follow-up publication reported on the multifaceted assessment of 412 MIBC tumors. Tumors assessed in the TCGA were all high-grade muscle-invasive tumors from chemotherapy-naïve patients. Pure urothelial carcinoma not otherwise specified (NOS) comprised the majority of samples; however, 52 tumors (13%) contained some element of variant histology. WES was performed to assess mutations (i.e., SNVs) and mutational signatures, while Affymetrix Genome-Wide Human SNP Array 6.0 (Thermo Fisher Scientific) was used to determine somatic copy number alterations (SCNAs). RNA sequencing allowed for expression-based molecular subtyping, and proteomic analysis was also performed. TCGA provided several insights into the molecular biology of bladder cancer. First, they confirmed the relatively high rate of somatic mutations, similar to melanoma and lung cancer, that has been seen in other pan-cancer studies [11]. This has important clinical implications given the United States Food and Drug

Administration (FDA) approval of pembrolizumab for tumors with TMB >10 mutations per megabase, regardless of the origin of malignancy. Second, using WES and unsupervised clustering of specific mutational data, the authors were able to identify multiple mutational signatures. The first are two apolipoprotein B mRNA editing enzyme, catalytic polypeptide (APOBEC)-like signatures, which collectively account for two-thirds of all SNVs in TCGA. Patients whose tumors demonstrate APOBEC signatures were noted to have higher TMB and better overall survival. Further, these mutations were clonal, suggesting that they occurred early in bladder cancer carcinogenesis. Several more recent studies have demonstrated that even within histologically normal urothelium, chromatin-modifying alterations are common and contribute to additional mutational burden [12, 13]. The second group of mutational signatures with clinical relevance involve *ERCC2*. ERCC2 is a helicase involved in nucleotide excision repair and is considered a DNA-damage response (DDR) gene. More specifically, ERCC2 unwinds DNA at sites of damage to allow for other proteins and enzymes to repair the damage. These mutational signatures offer insights into the pathogenesis of bladder cancer and highlight possible avenues for therapeutic intervention.

Another important finding from TCGA was the identification of RNA expression-based molecular subtypes, which have both prognostic and predictive potential. In general, these molecular subtypes paralleled those discovered in breast cancer tumors and included basal and luminal subtypes. While TCGA classified tumors into Clusters I–II (luminal) and III–IV (basal), other groups independently developed similar classification systems for muscle-invasive bladder tumors, and more recently, a consensus classification was proposed [14]. In general, luminal and basal tumors differ in appearance (papillary vs. nodular and flat), response to chemotherapy (less responsive vs. more responsive), and relative frequencies of various genomic alterations [15]. Despite the strengths and potential clinical utility of these classification systems, molecular subtype analysis has yet to be incorporated into routine clinical practice.

Predicting response to chemotherapy is an important clinical question and has been addressed in several studies from a genomics standpoint. Given the prevalence of ERCC2 mutational signatures from TCGA, the functional implications of *ERCC2* alterations have been evaluated and demonstrate a correlation with response to cisplatin-based chemotherapy [16, 17]. In another study comparing primary MIBC and secondary MIBC that progressed from non-muscle-invasive bladder cancer (NMIBC), patients with secondary MIBC had fewer *ERCC2* mutations, worse recurrence-free survival (RFS) and overall survival (OS) rates, and poorer response to neoadjuvant chemotherapy [18]. Identification of *ERCC2* as a potential biomarker predictive of chemotherapy sensitivity has led to two clinical trials testing bladder preservation in genomically selected patients with specific alterations, mainly in DDR genes. The Alliance 031701 trial (NCT03609216) is evaluating bladder preservation in highly selected patients with certain DDR alterations who demonstrate a complete clinical response after dose-dense gemcitabine and cisplatin neoadjuvant chemotherapy. The RETAIN trial (NCT02710734) evaluates a similarly selected cohort based on a partially overlapping set of genes and using a

different neoadjuvant chemotherapy regimen. Conversely, cisplatin chemotherapy resistance has been associated with *FGFR3* alterations and clonal mutations in integrin signaling pathway genes [8, 19].

Along with prognostic and predictive biomarkers, genomic sequencing of MIBC has also revealed a rich genomic landscape of actionable alterations found at clinically relevant frequencies. In one study of nearly 100 patients with high-grade bladder cancer (85% of which were MIBC), 61% had at least one clinically actionable alteration [20]. In addition to the previously mentioned neoadjuvant trials in patients with certain DDR alterations, two trials are evaluating *FGFR* inhibitors given the known frequency of *FGFR3* alterations in bladder cancer (NCT04197986 and NCT04294277).

Finally, the wide spectrum of histomorphologic subtypes of bladder cancer is being actively investigated to discover genomic drivers of variant histology. Some variants are enriched in specific alterations that are nearly pathognomonic. For example, plasmacytoid variant bladder cancer, which is known to present more commonly at a locally advanced stage with common positive surgical margins at cystectomy, almost always carries a deletion in *CDH1,* which encodes for the E-Cadherin protein [21]. Other variants, such as small cell carcinoma, resemble pure urothelial carcinoma with common *TERT* promoter mutations while also being enriched for *TP53* and *RB1* alterations [22]. Although these discoveries advance our understanding of the pathogenesis of bladder cancer with variant histology, they also expose actionable alterations that could expand treatment options in patients who are typically resistant to chemotherapy and have poor clinical outcomes.

In summary, significant sequencing data exist for patients with MIBC that aid in prognosis and treatment response prediction, although none have yet reached routine clinical practice in this disease state. Several clinical trials exist in the neoadjuvant and adjuvant space for genomically selected patients. Future work will continue to unravel the pathways that contribute to divergent differentiation and exposure of therapeutic vulnerabilities in these aggressive tumors.

Non-muscle-Invasive Bladder Cancer

Efforts in NMIBC have focused on developing molecular-subtype classifications, characterizing the genomic landscape and drivers, and attempting to correlate these findings with the diversity of clinical outcomes from this heterogeneous group of patients.

While molecular subtypes in MIBC have been independently derived by several groups and a consensus classifier has been proposed, subtypes in NMIBC are considerably less defined at this time. The most significant effort to date used RNA sequencing data to derive three molecular subtypes from a cohort of 460 patients with NMIBC and 14 patients with MIBC [23]. Class 1 represented largely luminal tumors with predictably frequent *FGFR3* alterations. Class 2 was also luminal-like, when compared with other classifiers for MIBC, but expressed more epithelial to

mesenchymal transition markers and had more frequent predicted mutations in *TP53* and DDR genes, such as *ERCC2*. Class 3 tumors were more basal-like but did not represent a subtype seen in TCGA. In terms of progression, class 1 and class 3 tumors generally had favorable outcomes, while class 2 tumors were significantly more likely to progress. While these subtypes provided some biological underpinning, they largely paralleled tumor grade and stage (e.g., classes 1 and 3 consisted of mainly low-grade Ta tumors and class 2 comprised the majority of the high-grade T1 tumors among the three groups) and have yet to be adapted clinically. In another study of 140 low-grade Ta and high-grade Ta tumors, whole genome sequencing clearly demonstrated a significantly more unstable genome in subgroup 2, which consisted mainly of high-grade Ta tumors [24]. Other groups have attempted to further identify subtypes in high-grade T1 tumors, which account for the majority of progression and cancer-specific mortality in NMIBC [25, 26].

The genomic landscape of NMIBC demonstrates *TERT* promoter and common chromatin-modifying gene alterations across all grades and stages, which are known early events in bladder cancer pathogenesis [27]. Notably, shifts in oncogenic drivers and/or targetable alterations can be observed with increasing grade and stage from low-grade Ta to high-grade Ta to high-grade T1. For example, *FGFR3* mutations, a known driver in low-risk tumors, decrease in frequency from greater than 80% in low-grade Ta to less than 40% in high-grade T1. Conversely, oncogenic drivers of aggressive disease, such as *TP53* and *RB1* were more common with the shift from low-grade to high-grade disease, and frequencies in high-grade T1 disease approached that of a TCGA MIBC comparator cohort, correlating with the clinical experience that at least a subset of these tumors had the potential for invasion and metastases.

Ongoing efforts are focused on identifying associations with recurrence and progression, as well as predictors of response to Bacillus Calmette-Guerin (BCG), as the failure of this treatment often results in therapeutic escalation to radical cystectomy. High-grade NMIBC tumors were found to have higher TMB which correlated with more frequent mutations in DDR genes, particularly *ERCC2* [27]. These findings were independently confirmed in a separate analysis consisting of 126 cases of high-grade NMIBC showing that TMB increased from low-grade NMIBC to high-grade NMIBC and that TMB and DDR alterations were positively correlated [28]. The association between TMB and response to BCG should be further explored, although theoretically, a higher mutational burden would result in a more robust response to an immunotherapy-based treatment (such as BCG) [27, 29]. On the other hand, significant associations between *ARID1A* alterations and BCG resistance were demonstrated in both studies, which is notable as these alterations could be a predictive biomarker of resistance to therapy and, in turn, potentially targetable.

In summary, molecular classification of NMIBC is based on comprehensive analyses of large patient cohorts but has yet to develop utility in clinical practice. NMIBC is of particular interest in terms of prognostic and predictive genomic biomarkers given the diversity of clinical outcomes that span from indolent yet recurrent low-grade tumors to quickly progressive and metastatic high-grade tumors. Finally, the lifelong invasive nature of surveillance for many patients with NMIBC

provides substantial motivation for advanced approaches of sequencing cell-free tumor DNA (ctDNA) in the urine.

Advanced and Metastatic Bladder Cancer

The genomic landscape of advanced and metastatic bladder cancer is similar to muscle-invasive disease but often influenced by the selective pressures of systemic treatment. In a study of 72 chemotherapy-resistant tumors and a subgroup of matched pre- and post-chemotherapy samples, few mutations were shared between the primary and metastatic tumors [8]. However, the divergence of primary and metastatic samples on WES occurred early in the evolution of these tumors indicating that this is an early event in the natural history of the disease. In a rapid autopsy series of multiple primary and metastatic sites from seven patients with both bladder and upper-tract cancer, discordance in mutations with potentially actionable mutations occurred in 30% of samples [30]. This finding highlights the potential importance of sequencing additional sites of disease as tumors become resistant to therapy, progress, or metastasize to additional sites, which can be addressed by advanced approaches such as ctDNA.

To date, the only FDA-approved targeted therapy for bladder cancer is the pan-*FGFR3* inhibitor, erdafitinib, which is approved for patients with locally advanced or metastatic disease that has progressed during or following platinum-containing chemotherapy [31]. Genetic testing for *FGFR2/3* alterations is indicated to identify patients for this treatment. No guidance is provided for indicating whether primary or metastatic samples should be tested for these alterations. To optimally select patients for targeted therapies, future studies will be required to determine whether known intra- and inter-tumoral heterogeneity results in inappropriate selection of candidates for treatment and which samples are ideal for genetic testing.

Germline Alterations

Epidemiologic studies have identified that approximately 30% of urothelial cancers have a heritable component [32]. However, while germline mismatch repair (MMR) variants have been associated with Lynch syndrome and the risk of urothelial carcinoma of the upper tracts, no clear associations with bladder cancer exist. Current efforts have focused on characterizing the landscape of germline alterations, evaluating the role for germline testing, and identifying clinically relevant implications of germline alterations in patients with bladder cancer.

Two large retrospective analyses have evaluated germline alterations in patients with urothelial carcinoma and identified similar rates and types of alterations [33, 34]. Using a panel of 77 cancer predisposition genes, one study found that up to 13.7% of patients had a pathogenic or likely pathogenic germline variant in a cohort

of 586 patients with urothelial carcinoma, majority of whom (79%) had bladder cancer [34]. In this study, the most frequently altered gene was *APC,* and the most frequently altered genes specifically with moderate or high penetrance were *BRCA2, MSH2, CHEK2, ERCC3, NBN*, and *RAD50*. In total, 83% of germline variants were in DDR genes. In the subgroup with clinically annotated data, patients with any moderate-/high-penetrance variant (n = 27) were more likely to be ≤45 years old (22% vs. 6%) and of Ashkenazi Jewish ancestry (41% vs. 14%) compared with patients with no moderate-/high-penetrance variants (n = 142). Importantly, one-quarter of patients with germline variants in this study would not have been referred for germline testing based on published guidelines, suggesting that current methods to identify patients with potentially hereditary bladder cancer are inadequate. A second study comprised a larger cohort (n = 1038) tested with an assay from Invitae (San Francisco, CA), which sequenced between 1 and 130 genes (median 42) [33]. Despite the heterogenous sequencing panel, similar results were obtained. Approximately 24% of patients carried a pathogenic germline variant, of which 18.6% were in actionable genes as defined by the NCCN. This study also found that germline DDR alterations accounted for the majority (78%) of germline mutations. Combined, these studies suggest that certain high-risk cohorts would benefit from germline testing, and future studies should strive to identify how to best select these patients.

Despite their prevalence, the clinical implications of germline variants have yet to be fully realized. Current germline analyses have focused on patients with advanced and metastatic disease, thereby limiting generalizability to patients with localized muscle-invasive and non-muscle-invasive disease. Additional studies are needed to delineate the role of germline testing in select patients with bladder cancer.

Liquid Biopsy

Circulating Tumor Cells and Cell-Free Tumor DNA

There is increasing interest in genomic analysis of circulating tumor cells and cell-free tumor genomic material in patients with bladder cancer [35]. These assays, often referred to as liquid biopsies, have multiple clinical applications from screening and diagnosis to risk stratification and surveillance. Analysis of circulating tumor cells requires the identification and isolation of intact tumor cells, which can be analyzed morphologically as well as from a molecular standpoint. Circulating cell-free tumor DNA (ctDNA), on the other hand, can be isolated from a blood draw and sequenced using NGS platforms. In metastatic bladder cancer, ctDNA has been shown to reproduce the genomic landscape of MIBC based on paired tumor tissue profiling and compared with an analysis of TCGA [36]. ctDNA may potentially overcome several limitations previously discussed that apply to bulk tumor tissue sequencing of bladder cancer. First, these assays may capture alterations that are

absent in bulk tumor sequencing given the known extent of intra-tumoral heterogeneity. Similarly, although many alterations in bladder cancer are thought to occur early in the development of tumors, inter-tumoral heterogeneity between primary tumors and metastatic sites may be better captured with ctDNA. This is especially possible in the setting of intervening targeted treatments (such as erdafitinib). Second, ctDNA can yield actionable genomic information in patients whose tumors are inaccessible without a high-risk invasive procedure (e.g., certain pulmonary metastases). Finally, serial ctDNA can be collected relatively simply, as this only requires a blood draw. Serial analysis of ctDNA provides the opportunity to evaluate response to treatment, guide additional therapies, and monitor resistance.

Monitoring of minimal residual disease after surgery is another potential application for liquid biopsies. In a prospective study of 68 patients with MIBC undergoing radical cystectomy, a primary tumor WES-informed customized ctDNA panel had a sensitivity and specificity of 100% and 98%, respectively, for the detection of recurrence after surgery [37]. Although this assay provides no targetable information to guide therapy selection, this sensitive assay could help guide adjuvant treatment in patients who are likely to have a recurrence. Further, ctDNA could be used to help guide treatment decisions in a variety of settings (e.g., early cystectomy, neoadjuvant chemotherapy, and consolidative surgery, among others) where the potential for over and undertreatment is substantial. Future studies are needed to better characterize the utility of ctDNA in these various disease states.

Urinary Cell-Free Tumor DNA

Analysis of ctDNA in the urine represents a logical strategy for the detection of bladder cancer. In a large analysis of 118 patients with bladder cancer and 67 healthy adults, Dudely et al. evaluated a novel hybrid-capture target enrichment strategy to sequence ctDNA from the discarded supernatant of urine samples [38]. *TERT* and *PLEKHS1* promoter mutations were the most commonly discovered alterations, and the concordance between mutations in tumor tissue and urinary ctDNA was between 67% and 73% and higher for clonal versus subclonal mutations. APOBEC mutational signatures were significantly more common in patients with bladder cancer compared with patients in the control group, suggesting the possibility of using this assay as a screening tool. Compared with cytology, urinary ctDNA had significantly higher sensitivity (83–93% vs. 14%) and equivalent specificity (96–97% vs. 100%). This assay was also practical in that 50 cc of urine could be stored at 4 °C for up to 7 days. Interestingly, urinary ctDNA may be more sensitive than plasma ctDNA. In one study of nearly 250 samples from 17 patients, urinary supernatant and urinary cell pellet had more frequent single nucleotide variants and higher mutant allele frequencies compared with plasma ctDNA [39]. Urinary ctDNA has many potential applications and prospective clinical trials are needed to better define its role in the management of patients with bladder cancer.

Guidelines and Practical Approach

Despite the accumulating data on the clinical applicability of genomic sequencing in bladder cancer diagnosis and management, there is no consensus approach and the major guidelines in urologic oncology do not yet uniformly recommend testing. The NCCN guidelines recommend molecular/genomic testing in patients with stages IIIB and IV disease to identify potential therapeutic targets and to screen for clinical trial eligibility [40]. The European Association of Urology (EAU) guidelines mention the potential future utility of genomic sequencing but no current indications in either the MIBC or metastatic bladder cancer guidelines. Finally, 2020 amended American Urological Association (AUA) guidelines for nonmetastatic MIBC discuss the potential of genomic prognostic and/or predictive biomarkers but do not recommend testing; the AUA NMIBC guidelines make no mention of genomic sequencing [41, 42].

At this time, genomic sequencing to guide clinical care should be limited to patients with stage IIIB or IV disease as per the NCCN guidelines. Genetic screening or testing in patients with earlier-stage disease should be limited to the clinical trial or prospective study setting. There are several practical considerations for testing in patients with advanced disease. First is the question regarding which tumor sites should be sequenced. While most targeted therapies and clinical trials will accept sequencing from any source, there are some studies that suggest genomic differences in key drivers between the primary and metastatic tumors. This would support sequencing of the metastatic material if that were available. Second, testing should be performed prior to initiation of therapy to reduce the influence of treatment on the results. Third, as previously discussed, assays that utilize matched normal samples will reduce error from germline mutations and clonal hematopoiesis of indeterminate potential. Finally, integration of genetic counselors to aid in the interpretation, education, and counseling of patients is of significant added value and likely to be more important over time as germline testing becomes more commonly indicated [43, 44].

Conclusions

There is increasing evidence to support the role of genomic sequencing in the management of patients with bladder cancer. In patients with MIBC, clinical and translational data have demonstrated that some DDR genes, specifically *ERCC2*, may confer cisplatin sensitivity, and current clinical trials are testing the role of genomic biomarkers to select patients for bladder preservation. Additional clinical trials are genomically selecting patients for adjuvant targeted therapies in patients at high risk of recurrence after radical cystectomy. In NMIBC, genomic analyses are helping to identify predictors of response to BCG and indications for more aggressive therapy in others. In advanced and metastatic disease, tumor genomic evolution is being investigated to understand the drivers of metastasis and how potential targeted therapies should be selected. Germline analysis may provide data to aid in risk

assessment for secondary malignancies and cascade testing in patients with alterations that confer an increased risk of hereditary cancers. In nearly all disease stages, analysis of ctDNA from blood and/or urine could revolutionize how samples are collected for analysis. As NGS technology advances and the cost of deeper and more broad sequencing falls, more complete sequencing (e.g., WES, WGS) may become routine.

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Chapter 12
Novel and Investigational Diagnostics: Liquid Biopsy and Beyond

Filipe L. F. Carvalho and Keyan Salari

Introduction

Clinical decision-making for the management of bladder cancer has been largely based on pathologic features of the primary tumor and imaging findings. However, the limited sensitivity of standard cross-sectional imaging techniques can potentially delay the detection of persistent, recurrent, and metastatic disease. Large-scale molecular profiling studies of bladder cancer have yielded important insights into disease biology, prognosis, and treatment strategies [1–3], and therefore, molecular characterization of bladder tumors is becoming increasingly important for clinical management. Dramatic advances over the past decade in next-generation sequencing technologies have enabled rapid and inexpensive genomic profiling of clinical tumor specimens to identify clinically actionable genomic mutations, structural alterations, and transcriptional signatures that provide prognostic information and predict response to therapies [3–5]. However, performing molecular characterization of tumors based on tissue biopsies requires an invasive procedure to obtain the specimen; tumor tissue biopsies can have complications such as bleeding [6] and can be uncomfortable for patients. In addition, tissue biopsies can often yield insufficient material for molecular profiling and are subject to sampling error with the potential to provide an unrepresentative molecular profile of the tumor. Particularly in the setting of advanced disease, where different metastatic sites of disease can harbor distinct tumor clones or subclones, tissue sampling from one metastatic site can significantly bias molecular assessment of the patient's cancer and its genomic heterogeneity [7].

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Given the inherent limitations of tissue-based biomarkers, there has been growing interest in developing noninvasive methods for robust tumor molecular characterization using patient plasma or serum specimens. Plasma and serum contain variable amounts of tumor-derived nucleic acids, proteins, metabolites, and even intact tumor cells; the process of detecting these tumor macromolecules and cells from a patient blood sample is known as a liquid biopsy (Fig. 12.1). Multiple liquid biopsy platforms have been developed to isolate and analyze tumor-derived circulating tumor cells (CTC), cell-free circulating tumor DNA (ctDNA), and circulating tumor RNA (including microRNA and exosomal RNA). The relative ease of obtaining a blood sample compared to a tumor biopsy, as well as less potential for sampling error [8], has led to growing enthusiasm for liquid biopsy approaches to many malignancies including bladder cancer. Potential applications of liquid biopsy-based assays for bladder cancer include the detection of disease at initial diagnosis or recurrence, detection of minimal residual disease after treatment, identification of targetable genomic alterations, and monitoring of disease evolution and treatment response through serial assessments.

Here, we will describe the current landscape of liquid biopsy approaches that have been explored to date in bladder cancer, current barriers to clinical implementation, and future directions.

Circulating Tumor Cells

Circulating tumor cells (CTC) represent intact and sometimes viable cells that can be isolated from blood based on their cell surface molecules and physical characteristics such as size or electrical charge [9, 10]. The CellSearch system is the first (and currently only) FDA-approved platform for clinical use to detect CTCs in peripheral

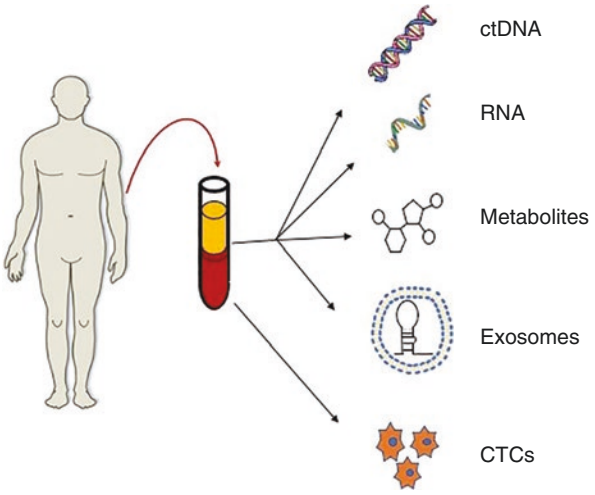

Fig. 12.1 Biomarkers for bladder cancer detection and monitor response to therapy. Circulating tumor cells (CTC) can be isolated based on size, surface makers, or cell density. Advances in sequencing technologies and spectrometry allow the detection of specific mutations present in circulating tumor DNA (ctDNA), RNA, exosomes, and cancer metabolites

blood; it was approved in 2004 and uses immunomagnetic enrichment to capture cells based on the epithelial cell membrane marker EpCAM [10]. However, a number of other technologies have also been studied to refine the enrichment, enumeration, recovery, and characterization of CTCs and, thus, the optimal protocols for sample collection and processing continue to evolve.

Early studies demonstrated that urothelial cancer cells could be detected in peripheral blood of patients with metastatic bladder cancer (44–75% detection rate [10–12]), with a higher number of detectable CTCs being associated with increased metastatic burden [12]. While initial studies reported the absence of detectable CTCs in patients with clinically localized disease, CTCs were detected in preoperative blood samples in nearly one-quarter of patients with nonmetastatic disease undergoing radical cystectomy, where CTC positivity remained a significant independent predictor of disease recurrence and cancer-specific and overall mortality after adjusting for effects of standard clinicopathologic features [13]. Even in the setting of high-risk NMIBC, two studies evaluating a total of 257 patients with T1 high-grade urothelial carcinoma detected CTCs in the peripheral blood of 20–28% of patients prior to TURBT; the presence of CTCs in this setting was significantly associated with time to first recurrence and progression-free survival [14, 15].

While the majority of CTC studies have utilized the CellSearch platform, the reliance on positive selection for the EpCAM cell surface protein may limit its sensitivity. Using a novel selection-free method to enumerate and characterize CTCs in 38 patients across a range of stages, Chalfin et al. were able to detect CTCs in 25%, 58%, and 67% of patients with T1 NMIBC, MIBC, and metastatic disease, respectively [16]. Further, these CTCs exhibited phenotypic diversity of cell size and EpCAM expression; EpCAM-positive CTCs were not detected in any patient with NMIBC and were present in only two (17%) patients with MIBC. Thus, EpCAM-negative CTCs were detected in NMIBC and MIBC patients that would have been missed with the CellSearch platform.

Beyond evaluating the simple presence of CTCs for potential prognostic value, molecular characterization of these individual cells has also been explored. Coupling CTC isolation with RT-PCR or next-generation sequencing has enabled detailed single-cell analysis of CTC gene expression, where studies have shown prognostic relevance of *EGFR*, *UPKII*, *TNC,* and Survivin mRNA transcripts detected in or on CTCs [17–19]. In other malignancies, CTC-derived transcriptional analysis has been used to identify targeted therapy-related alterations or signatures associated with drug response or resistance; for example, PD-L1 expression on CTCs has been found in breast and head and neck cancers and may serve as a predictive biomarker for immune checkpoint inhibitor response [20, 21]. Ongoing clinical trials of bladder cancer patients incorporating CTC analysis before and after treatment with immune checkpoint inhibitors (e.g., NCT02978118) will help provide insight as to whether CTC-based assessment of immune checkpoint proteins can predict response to immunotherapies better than tissue-based assessment.

One fundamentally unanswered question with respect to CTCs is their biological role, which remains poorly understood, especially in patients with NMIBC that are at risk of progression to MIBC but have low rates of metastasis. Prior studies in

prostate cancer showed that 70% of men with clinically localized prostate cancer undergoing radical prostatectomy have tumor cells in the bone marrow prior to surgery [22]. In the same study, almost 60% of these patients with tumor cells in the bone marrow did not have biochemical recurrence after surgery, suggesting that the majority of these CTCs do not have the capacity to generate metastasis. If CTCs do not represent the cells responsible for metastatic seeding, then this challenges the rationale for making clinical treatment decisions based on the molecular characterization of these cells. Particularly in cases of discordant expression between the primary tissue and CTCs (e.g., Rink et al. showed discordance between HER2 expression on CTCs and HER2 gene amplification status in primary tumors in 23% of cases [13]), it is unclear which biospecimen provides the most relevant information as a predictive biomarker. Finally, while CTCs offer the ability to molecularly characterize viable tumor cells, their relatively low abundance (often only 1–10 CTCs detectable per 10 mL blood specimen) may limit their sensitivity compared to liquid biopsy approaches with more abundant tumor-derived materials such as cell-free circulating tumor DNA [11].

Circulating Tumor DNA

Cell-free circulating tumor DNA (ctDNA) represents tumor-derived fragments of DNA that have been shed into the circulation. The precise mechanism of ctDNA release is unclear. ctDNA is typically found as double-stranded DNA fragments 150–200 base pairs in length and has a half-life of 1.5–2.5 hours in the bloodstream [23, 24]. While cell-free DNA found in circulation is often derived from unrelated normal tissues, the fraction of cell-free DNA derived from the tumor in cancer patients varies greatly from <1% to 90% [25]. Advances in next-generation sequencing and droplet digital PCR (ddPCR) technologies have allowed for the detection of very low-abundance tumor DNA in biospecimens such as blood and urine. Whereas conventional PCR amplifies all nucleic acids in a patient sample together in one reaction, ddPCR entails separating a patient sample into a large number of partitions (using oil-based droplets to encapsulate individual nucleic acid molecules) and carrying out the amplification reaction in each partition individually. This methodology allows for the detection and quantification of target nucleic acids in low abundance (e.g., mutations present in ctDNA) that would otherwise be undetectable by conventional PCR. With these technological advances, ctDNA has been evaluated for use in bladder cancer early detection of recurrence, identification of tumor mutations for targeted therapies, and detection of minimal residual disease after treatment.

Early studies indicated that patients with urothelial carcinoma had among the highest fractions of detectable ctDNA across several cancer types [26] and demonstrated that ctDNA could be identified in patients with noninvasive disease and before disease progression [27]. Using a ddPCR assay specifically targeting *FGFR3* and *PIK3CA* hotspot mutations, Christensen et al. demonstrated that increased levels of *FGFR3* and *PIK3CA* mutated tumor DNA in the urine of patients with NMIBC

or in the plasma from patients who underwent cystectomy were associated with disease progression and metastasis [28]. In the most comprehensive prospective study of ctDNA in bladder cancer, the same investigators assessed ctDNA by ultra-deep sequencing of plasma DNA using a panel of patient-specific mutations longitudinally in a cohort of 68 MIBC patients who were receiving neoadjuvant chemotherapy before cystectomy [29]. In this study, detection of ctDNA at diagnosis before neoadjuvant chemotherapy was significantly associated with worse recurrence-free survival and overall survival. The detection of ctDNA at this early time point was, therefore, a strong prognostic factor for the long-term clinical outcome after chemotherapy and cystectomy. In addition, detection of ctDNA after radical cystectomy was also significantly associated with disease recurrence and worse overall survival. Most significantly, ctDNA status during disease surveillance after cystectomy was highly prognostic, with an overall recurrence rate of 76% in ctDNA-positive patients and 0% in ctDNA-negative patients. Notably, ctDNA status after cystectomy was the strongest predictor of recurrence-free survival in multivariate analysis including any other predictive factor such as clinical lymph node status and pathologic downstaging. Finally, detection of ctDNA during surveillance anticipated radiographic evidence of tumor recurrence with a median lead time of 3 months. These findings taken together support the role of ctDNA as a prognostic biomarker of minimal residual disease, response to chemotherapy, and disease recurrence with a positive lead time over radiographic imaging.

Several studies have explored the ability of ctDNA to identify clinically actionable genomic alterations in patients with metastatic bladder cancer as a practical and reliable alternative to tissue biopsies. Vandekerkhove et al. demonstrated in a cohort of 51 patients with locally advanced/metastatic bladder cancer robust detection of targetable alterations in MAPK/ERK or PI3K/AKT/mTOR pathways, including amplification of *ERBB2* (20% of patients) and activating hotspot mutations in *PIK3CA* (20%) [30]. This application of ctDNA has raised the question of whether genomic alterations detected by ctDNA are concordant with those present in tumor tissue. It can be technically challenging to discriminate between normal DNA and tumor DNA in the blood due to low tumor DNA, especially in early-stage and minimal residual disease settings, which can lead to potential false negatives. Additionally, multiple factors such as different gene panels, platforms, and bioinformatic methods can contribute to this variability. A recent study compared ctDNA profiles from 104 patients with metastatic bladder cancer to matched patient tumor tissue and revealed that ctDNA analysis faithfully reproduces matched tissue-based genomic profiles with a high mutation concordance of 83.4% [31]. Further, 90% of mutations were identified across serial ctDNA samples, whereas the concordance for serial tumor tissue was significantly lower. Therefore, ctDNA appears to be a reliable surrogate for tumor tissue, but additional prospective clinical studies are needed to evaluate the utility of ctDNA profiling to guide treatment selection.

As an indicator of minimal residual disease, ctDNA detection is currently being integrated into clinical trials with the goal of better selecting patients for adjuvant therapy in an effort to reduce overtreatment and treatment-related toxicity. One such trial (NCT04138628) is currently recruiting patients following radical cystectomy

for MIBC to investigate the response rate and oncological outcome of the immune checkpoint inhibitor atezolizumab administered at the time of "biochemical relapse" defined by the detection of ctDNA. The investigators hypothesize that early initiation of immunotherapy in high-risk ctDNA-positive patients will result in better response rates and improved survival compared to treatment prompted by radiographic recurrence on conventional imaging.

Circulating Tumor RNA

In addition to CTCs and ctDNA, it is also possible to isolate circulating tumor RNA present in the bloodstream. RNA can be transcribed from protein-coding regions of the genome and translated into protein (i.e., messenger RNA and mRNA) or transcribed from noncoding regions to form noncoding RNAs important in a variety of cellular functions including gene regulation. microRNA (miRNA) are small noncoding RNA molecules with an average length of 22 nucleotides. Noncoding RNA molecules larger than 200 nucleotides are called long noncoding RNA (lncRNAs). Whereas mRNA can be quickly degraded, miRNAs and lncRNAs are often protected by a protein complex and can be packaged inside small extracellular vesicles, called exosomes, that preserve RNA integrity and prevent degradation in liquid biopsy specimens. While the half-life of unprotected cell-free mRNA in the plasma is short (<15 seconds) [32], noncoding RNAs bound to proteins or inside exosomes have prolonged half-lives of 1.5–13 hours [33]. These properties make noncoding RNAs, in particular those packaged within exosomes, an appealing potential biomarker in the blood or urine of bladder cancer patients [34].

Studies have shown microRNAs in serum exosomes can accurately distinguish patients with genitourinary malignancies, such as kidney and bladder cancer, from healthy individuals [35, 36]. Adam and colleagues developed a serum miRNA-based classifier based on the most differentially expressed miRNAs between bladder cancer patients and healthy controls, which achieved 89% accuracy for detecting the presence or absence of bladder cancer and 100% accuracy for distinguishing MIBC from healthy controls [35]. This study also identified the most diagnostically useful miRNAs to be miR-541, miR-200b, miR-566, miR-487, and miR-148b, which had significantly higher abundance in the plasma of bladder cancer patients compared with controls. Follow-up genome-wide miRNA sequencing of serum from patients with bladder cancer validated some of these miRNAs and their role in early detection [37]. A 6-miRNA panel composed of miR-152, miR-148-3p, miR-3187-3p, miR-15b-5p, miR-27a-3p, and miR-30a-5p had significantly higher sensitivity than urine cytology in detection and stratification by tumor stage. Higher miR-152 levels and lower levels of miR-3187-3p in the serum were significantly associated with higher tumor stage and reduced recurrence-free survival in patients with NMIBC.

Beyond serving as a biomarker, select noncoding RNAs have also been found to have an important role in bladder cancer biology. For example, *in vitro* studies using bladder cancer cell lines have implicated miR-200b and other members of

the miR-200 family in epithelial-to-mesenchymal transition, a cell state that is crucial for invasion and metastasis, and sensitivity to targeted therapy with EGFR inhibitors [38]. Further, exosomal miRNA-148b has been shown to be associated with resistance to chemotherapy in patient-derived bladder cancer cell lines and mouse xenografts [39]. Finally, the lncRNA *LNMAT2* has been found to be enriched in exosomes from bladder cancer patients, promote lymphangiogenesis *in vivo* and *in vitro*, and correlate with lymph node metastasis [40].

Taken together, these studies show enormous promise regarding liquid biopsy approaches evaluating miRNAs, lncRNAs, and exosomes in bladder cancer patients. Further investigation is warranted to evaluate the utility of these circulating RNA-based biomarkers in prospective studies.

Metabolites

Perturbations in several metabolic pathways including glycolysis, the Krebs cycle, fatty acid oxidation, and amino acid metabolism are present in bladder cancer. These alterations generate an excess of metabolites that can be detected in the urine or serum of bladder cancer patients and serve as a fingerprint of the disease state [41]. In preclinical studies, high-grade compared to low-grade bladder cancer cell lines have shown significantly lower levels of fatty acids and higher levels of the amino acid metabolites aspartic acid, leucine and methionine, and ammonia, which may reflect a metabolic shift to different energy pathways to meet the higher metabolic demands of cell proliferation and invasion [42]. Advances in nuclear magnetic resonance (NMR) spectroscopy technology have allowed the detection of metabolomic profiles in the serum of patients with bladder cancer, and NMR-based metabolomic analysis has been able to distinguish low- from high-grade tumors based on the differential abundance of amino acid and glycolytic metabolites [43]. Specifically, patients with high-grade tumors were found to have significantly lower levels of the amino acids tyrosine and phenylamine. The clinical and therapeutic implications of these metabolic differences in high-grade tumors have not been completely elucidated yet. These promising results show that serum metabolites have the potential to be used as noninvasive biomarkers. Standardized protocols to detect consistent metabolic profiles are needed to evaluate this technology in larger cohorts of patients and validate these findings as well as explore their association with other clinical outcomes of interest.

Other Serum-Based Biomarkers

Whereas the aforementioned liquid biopsy methods enable molecular characterization of the tumor based on tumor-derived materials released into the bloodstream, more traditional serum-based biomarkers have also been explored in bladder cancer.

Pretreatment neutrophil-to-lymphocyte ratio (NLR)) is a marker of systemic inflammation that has been associated with adverse outcomes in a variety of cancer types. In a cohort of 899 patients undergoing radical cystectomy with a median of 10.9 years follow-up, an elevated pretreatment NLR was associated with increased risk of locally advanced disease, disease recurrence, and cancer-specific and all-cause mortality [44]. In a separate study of 122 predominantly low- and intermediate-risk NMIBCs, an elevated NLR was associated with high tumor grade, recurrence of NMIBC, and progression to MIBC [45]. While the exact biological mechanism underlying the relationship between high NLR and adverse oncologic outcomes is unclear, the studies to date suggest that serum NLR may be a practical and inexpensive prognostic biomarker for pretreatment risk stratification including consideration for intravesical therapy and neoadjuvant therapy in the NMIBC and MIBC settings, respectively.

Carbohydrate antigens (CA) have been successfully used as serum tumor markers for detection and response to therapy of several cancer types (e.g., CA 19-9 for pancreatic cancer and CA-125 for ovarian cancer). In bladder cancer, these serum tumor markers have been found to be independent predictors of response to neoadjuvant chemotherapy and oncologic outcomes. Ahmadi and colleagues showed patients with locally advanced bladder cancer had significantly higher serum levels of CA 19-9 prior to radical cystectomy compared to patients with organ-confined disease [46]. Elevated CA 19-9 prior to radical cystectomy was also associated with significantly higher risk of disease recurrence and mortality within 3 years of surgery. Using a panel consisting of CA 19-9, CA-125, and CEA, Bazargani et al. evaluated 337 patients undergoing radical cystectomy and found elevated serum tumor markers were associated with worse recurrence-free and overall survival [47]. Among the subset who underwent neoadjuvant chemotherapy, post-chemotherapy marker normalization was associated with a longer time to progression and significantly better recurrence-free and overall survival compared to patients with persistently elevated markers [47]. Similar to NLR, serum tumor markers are easily obtainable and inexpensive, but larger prospective studies are still needed to validate the above associations and define the role of serum tumor markers in clinical decision-making.

Conclusion

Liquid biopsy assays have enormous clinical potential, and in the past decade, tremendous progress has been made to develop novel technologies to detect a variety of tumor-derived materials in the circulation of cancer patients. While there is great enthusiasm for liquid biopsy approaches in bladder cancer, a number of challenges must still be overcome before they can be incorporated into routine clinical decision-making.

First, the biological relevance of each tumor-derived material must be better understood. In a disease with significant genomic heterogeneity, it is unclear

whether ctDNA fragments are derived from representative viable tumor cells or represent residual DNA from dying cells that are irrelevant to the metastatic potential of the tumor. Similarly, the finding of CTCs present in patients with NMIBC who do not go on to develop metastasis calls into question whether CTCs are relevant to metastatic seeding or are simply incidental cells that lack the ability to generate metastases. While a biological or mechanistic understanding is not strictly necessary for a prognostic biomarker, a liquid biopsy test must interrogate material from the most relevant tumor cell populations in order to reliably report the presence or absence of actionable genomic alterations that might impact therapeutic decisions. Second, as with all biomarker development efforts, the analytical validity of liquid biopsy assays must be improved to overcome the different methodologies for specimen collection, storage, processing, and analysis. All these steps can influence the degradation of sensitive molecules such as RNA or metabolites and introduce technical artifacts. Several consortia, such as the European Liquid Biopsy Society and the US-based BloodPAC project, are partnering with academic and industry experts to standardize methods to make liquid biopsy assays more reproducible and accelerate their translation to the clinic. Finally, large prospective clinical studies are needed across the spectrum of relevant clinical contexts to establish the clinical utility of each liquid biopsy test. As these efforts mature, we will likely witness an expansion in the armamentarium of validated liquid biopsy tests available for bladder cancer patients across various clinical contexts of the disease.

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Index

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